

Crossbreeding effects on some semen and litter traits in rabbits

By

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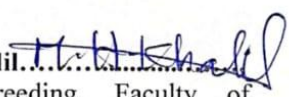
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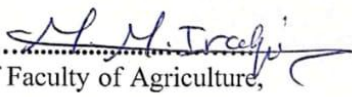
CROSSBREEDING EFFECTS ON SOME SEMEN AND LITTER TRAITS IN RABBITS

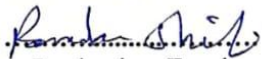
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1. INTRODUCTION

Crossbreeding is usually used to improve the overall production efficiency by using some breeds which have high genetic merit for different economic traits. In crossbreeding experiments performed in rabbits, most of the Egyptian and non-Egyptian studies (e.g. **Piles et al., 2004; Abou Khadiga et al., 2008; Iraqi et al., 2008; Youssef et al., 2009; Khalil and Al-Homidan, 2014**) showed significant direct additive and maternal genetic effects along with significant heterotic effects on body weights and gains at different ages. Until now, few studies have surveyed candidate genes for reproduction and growth traits in rabbits (**Peiró et al., 2008; Merchán et al., 2009; García et al., 2010**).

The wide use of artificial insemination in the rabbit farms has raised the interest in evaluating the rabbit semen, in order to estimate the genetic potential of the seminal doses in breeding programs. Earlier reports on semen quantity and quality of crossbred males from sire lines have showing a change in rabbit meat production schemes by using crossbred males in the terminal cross instead of purebred males (**Brun et al., 2002; Al-Sobayil and Khalil, 2002**). **Khalil et al. (2007)** indicated that the crossbred bucks were associated with heterotic effects in ejaculate volume (11.6%; $P < 0.05$), sperms concentration (10.5%; $P < 0.05$), percentages of motile sperms (9.8%; $P < 0.05$) along with a reduction in percentages of abnormal sperms (10.8%) and dead sperms (23.5%; $P < 0.05$). **El-Tarabany et al. (2015)** in crossbreeding experiment between New Zealand White

with Flander found that there were positive estimates of direct heterosis for ejaculate volume, mass motility, individual motility and concentration of sperms.

The candidate genes have successful approaches in identifying several DNA markers associated with production traits in rabbits. The *GH* gene alters the metabolism of carbohydrates, proteins and lipids and promotes postnatal growth of mammals through direct or indirect effects on numerous tissues (**Fontanesi et al., 2012**). The technique of polymerase chain reaction (PCR) based on restriction fragment length polymorphism (PCR-RFLP) was recently used by **Amalianingsih and Brahiantiyo (2014)** and **Hussein et al. (2015)** for analysing single nucleotide polymorphisms (SNP) of the *GH* gene in rabbits where a significant association between the SNP and marketing weight was detected. **Abdel-Kafy et al. (2016)** recording the association between the (C>T) SNP of *GH* gene with growth and reproductive traits in rabbits populations, the heterozygote genotype (T/C) was significantly ($P<0.05$) associated with heavy body weight at 8 weeks and daily gain through 5-8 week interval. Using nucleotide sequence analysis, **El-Sabrout and Aggag (2017)** examined DNA parts of six growth genes: growth hormone (*GH*), growth hormone receptor (*GHR*), melanocortin 4 receptor (*MC4R*), phosphoglycerate mutase (*PGAM*), myostatin (*MSTN*), and fibroblast growth factor (*FGF*) for two rabbit lines (V line and Alexandria) to investigate the associations between SNP of these genes and body weight at marketing.

GH gene mutations are described in several species, however, the studied concerning its variability and associations with economic

traits in rabbits are scarce. Therefore, the aims of the current study were: (1) to estimate genetic parameters for growth and semen traits of rabbits in all genetic groups. (2) to estimate crossbreeding effects (i.e. direct additive, maternal additive and heterotic effects) on some growth traits and semen traits in a crossbreeding experiment between APRI line (A) and Moshtohor line (M), (3)) to use the PCR-RFLP as a fast efficacy technique and low cost method to genotype the C >T SNP located in the promoter region of GH gene in Moshtohor and APRI lines and their parents of Spanish V line and Gabali rabbits and (4) to detect the associations between (C>T) SNP genotypes of *GH* candidate gene, both of growth traits and semen traits in rabbits.

2. REVIEW OF LITERATURE

2.1 Synthetic lines of rabbits produced in Egypt:

Crossbreeding is looked as one of the fast tools available to produce synthetic lines of rabbits (**Oseni et al., 1997; Soller, 1998**). Also, it is a way to improve productive and reproductive traits, where it combines the variation between breeds to make new breeds (**Porter, 1993**), as in the case of crossbreeding experiments practiced between local breeds and foreign breeds to construct synthetic lines. In these experiments, synthetic lines have been decided to combine required genes for commercial important traits (**Brun and Baselga, 2010**).

Since 1998 when the Spanish V line rabbits were imported from Spain to Egypt, three selection experiments were done aiming to improve the ability of V line rabbits and their crosses for production and reproduction under hot climatologic conditions (**Khalil and Baselga, 2002**). APRI was the first synthetic rabbit line sophisticated as a maternal line from crossing V line with Baladi Red (**Youssef et al., 2008**). Alexandria was a synthetic paternal line originated in Alexandria University, from crossing V line with Baladi Black rabbits (**El-Raffa, 2007**). Then, a new synthetic multi-purpose line was developed in Benha University, called Moshtohor line, originated from crossing V line with Sinai Gabali rabbits (**Iraqi et al., 2007**).

2.1.1 Provenance and advantages of V line rabbits:

The Spanish V line was developed in 1983 in Valencia (Spain), as a maternal synthetic line by crossing the progeny of four specialized maternal lines. This line is medium in size and has white color. It has been selected for litter size at weaning (**Estany et al., 1989**). This line has been selected in places where the environment is closer to a hot environment than to a temperate one and it was tested in some hot climate countries. Some endeavors already were done and V line has proved to be advantageous to standard Californian and New Zealand White in Egypt (**Khalil, 2010**).

The V line rabbits genetically selected for more than 35 generations and were introduced to various countries (as alive animals or as frozen embryos) by using new bio-techniques and by stratifying selection and crossbreeding programs with local rabbits. This line was widely dispensed in some hot countries of the world like Saudi Arabia and Egypt (**Khalil, 2010**). V line displayed higher direct and maternal genetic effects for litter and locational performances and for post-weaning growth and carcass traits than native rabbits. In 1998, two selections experiments were performed with Baladi Black and with Baladi Red. In 2000, V line rabbits were crossed with Sinai Gabali rabbits at Benha University (**Khalil and Baselga, 2002**).

2.1.2 Provenance of Gabali rabbits:

There are two breeds of rabbits in Egypt bearing the name 'Gabali'. The first one was originated in the western desert of the north Mediterranean coast while the second one was originated in

Sinai desert. The two strains seem to be acclimatized to the desert conditions. The color of these rabbits is fundamentally grey. In 1992, the western coast rabbits were developed by the Desert Research Center, Ministry of Agriculture, Egypt through a project in Marriott (North Western coast of Egypt). Another project funded by the Ministry of Agriculture, started in 1994 in the Faculty of Agriculture at Moshtohor, Zagazig University, Egypt and this project was started for characterizing Sinai Gabali rabbits.

Gabali rabbits were raised by the Bedouins for their food. Moreover, they were reared in areas of western Giza governorate by some breeders. Gabali breed is a medium sized breed and it was bred mainly for meat because it is characterized by heavy litter weight at weaning and high ability to afford harsh environmental conditions and their high resistance for many diseases (Afifi et al., 2002). According to the previous advantages, Gabali rabbits were used in some crossbreeding experiments with different exotic breeds imported to Egypt (e.g. V line, New Zealand White and Californian) to ameliorate doe reproductive performances, milk production, post-weaning growth and carcass traits (Iraqi et al., 2008).

2.1.3 Provenance of Moshtohor rabbits:

Moshtohor rabbits (M line) is an Egyptian multipurpose synthetic line produced in Rabbitry of Faculty of Agriculture, Moshtohor, Benha University, from crossing the Spanish V line does (V) with the Egyptian Sinai Gabali bucks (G) to produce $\frac{1}{2}G\frac{1}{2}V$ cross (Iraqi et al., 2008). This line was being sophisticated to be convenient in hot climate in Egypt. This synthetic line has the

adaptability to reproduce efficiently in different systems of hot climate and it has also good ability to grow profitably in hot climate areas (**Khalil, 2010**). This cross was done to ameliorate litter traits and growth traits globally and it was selected for five generations for litter weight at weaning. The rabbits of this line are moderate in size and they have different colors ranging from yellowish-brown to gray.

2.1.4 Provenance of APRI line:

APRI line was established in the Animal Production Research Institute, Agriculture Research Centre, Ministry of Agriculture, using V line and Baladi Red rabbits as founder breeds. The V line was maintained at the stations of Sakha and Gimmiza and it was selected for litter weight at weaning. The first step of synthesis of APRI rabbits was to get F₁ coming from crossing Baladi Red bucks with V line does, then selecting for litter weight at weaning for three generations. The animals were called APRI line and the ratio of genes in this line is 50% from Red Baladi and 50% from line V. APRI line was kept at Sakha station (**Youssef et al., 2009**).

2.2 Crossbreeding effects on growth traits:

2.2.1 Direct additive genetic effects (G^I):

The estimates of G^I for growth traits are summarized from literature and cited in **Table 1**. Most of the Egyptian studies (e.g. **Khalil and Khalil, 1991; Abdel-Ghany et al., 2000a; Ibrahim et al., 2008; Youssef et al., 2009**) showed significant direct additive genetic effects on body weights at 4, 6 and 8 weeks of age. **Khalil and Khalil (1991)** reported significant estimates of G^I for body

weight at 12 weeks of age and non-significant for post-weaning body weight at 4 and 8 weeks. **Abdel-Ghany et al. (2000a)** noted that direct additive effects from crossing New Zealand White with Baladi Red or Baladi Black were consistently in favour of Baladi Red or Baladi Black for post-weaning body weights and gains. **Ibrahim et al. (2008)** showed that direct additive genetic effects were significant for growth traits at 4, 8 and 12 weeks of age in the cross of $\frac{3}{4}$ Gabali and $\frac{1}{4}$ New Zealand White. **Youssef et al. (2009)** found that direct additive effects between V line and Baladi Red rabbits were in favour of V line rabbits reaching 15.0% at 4 weeks and 13.3% at 12 weeks and these effects for daily body gains were significant during most age intervals reaching 35.7% at the interval of 10–12 weeks.

In non-Egyptian studies, V line was crossed with another maternal line (A line) and one paternal line (R line) and body weights and gains from 32 to 60 day were the least in V line and A line and their crosses, while R-line and its cross were the greatest (**Ea, 1999**). **Piles et al. (2004)** reported significant estimates of direct additive effects for body weight at 60 days in crossing C line and R line rabbits in Spain. **Ouyed and Brun (2008b)** in crossing New Zealand White with Californian reported significant direct effects in favour of New Zealand White for 63-d body weight. **Khalil et al. (2005)** in crossing V line with Saudi Gabali reported significant estimates of additive effects for most body weights and daily weight gains studied. Also, the direct additive effects were significantly ranging from 2.7 to 8.1% for body weights and 3.8 to 13.1% for daily weight gains as reported by **Khalil and Al-Homidan (2014)**.

Table 1: Percentages of direct additive effects (G^I %) for body weights and daily weight gains as cited in the Egyptian literature

Reference	Breeds or strains ⁺ used in crossbreeding	Body weight		Daily weight gains	
		Age (week)	G^I %	Age interval	G^I %
Abdel-Ghany et al. (2000a)	New Zealand White x Baladi Black	6	12	--	--
		8	1.9	6-8	28.1
		10	1.05	8-10	-15.7
		12	0.76	10-12	-9.8
		14	-3.17	12-14	-13.5
		16	-3.01	14-16	-11.2
Khalil and Afifi (2000)	Gabali x New Zealand White	4	-1.3	--	--
		6	4.1	--	--
		8	-0.8	--	--
		10	1.3	--	--
		12	-6.1	--	--
Khalil et al. (2002)	Gabali x V line	6	14.6	--	--
		8	16.3	--	--
		10	7.2	--	--
		12	9.2	--	--
Abou Khadiga et al. (2008)	V line x Baladi Black	4	52.1	4-8	48.5
		8	56.4	8-12	38.0
		12	54.8	4-12	41.0
Iraqi et al. (2008)	Sinai Gabali x V line	4	5.1	--	--
		8	6.5	4-8	11.3
		12	5.3	8-12	21.4
Youssef et al. (2009)	Baladi Red x V line	4	-15	4-6	20.9
		6	-2.7	6-8	-8.3
		8	-4.2	8-10	-17.7
		10	-7.4	10-12	-35.7
		12	-13.3	4-10	-2.8
		--	--	4-12	-11.5
Hekil et al. (2011)	New Zealand White x Californian	4	5.5	4.6	3.7
		6	4.1	6-8	-5.4
		8	3.4	8-10	-0.97
		10	2.4	10-12	-0.23
		12	2.3	4-12	-3.44

The first character of breed used as a sire and the next character used as a dam

2.2.2 Maternal additive effects (G^M):

The estimates of G^M in most of the Egyptian studies for growth traits are summarized from the literature and have shown in **Table 2.** (e.g. **Khalil and Afifi, 2000; Abdel-Ghany et al., 2000a; Khalil and Al-Homidan, 2006; Abou Khadiga et al., 2008; Iraqi et al., 2008; Hekil et al., 2011**). These estimates showed significant maternal effects on body weights at 4, 6 and 8 weeks of age. In this concept, the maternal additive effects on body weight and daily gain were significant in preference of New Zealand White at all ages studied (**Abdel-Ghany et al., 2000a**). **Ibrahim et al. (2008)** showed that the maternal additive effects on body weights at 8 and 12 weeks were significant ($P<0.01$). **Iraqi et al. (2008)** found that Gabali breed was significantly superior in maternal additive over the V line for body weights at 8 and 12 weeks of age and daily weight gains at intervals of 4-8 and 8-12 weeks ($P<0.01$). Maternal genetic effects were found to be positive and significant, in favor of line V dams for body weights at 8 and 12 weeks of age (**Abou Khadiga et al., 2008**). **Hekil et al. (2011)** found that maternal additive effects were in favour of New Zealand (N) breed over Californian (C) for most body weights studied, i.e. N- dammed rabbits were heavier than those of C-damed at most ages studied.

In non-Egyptian studies, **Medellin and Lukefahr (2001) and Piles et al. (2004b)** showed non-significant maternal effects on body weights at different ages. **Khalil et al. (2005)** in crossing V line with Saudi Gabali reported significant estimates of maternal effects for most body weights and daily gains. **Ouyed and Brun (2008b)** in

crossing Californian and New Zealand White found that maternal additive effects on 9 weeks body weight were generally non-significant. Also, the maternal effects were significantly ranging from 2.5 to 7.1% for body weights and 2.9 to 9.3% for daily weight gains as reported by **Khalil and Al-Homidan (2014)**.

Table 2: Percentages of maternal effects (G^M %) for body weights and daily weight gains as cited in the Egyptian literature

Reference	Breeds or strains ⁺ used in crossbreeding	Body weight		Daily weight gains	
		Age (week)	G^M %	Age interval	G^M %
Afifi et al. (1994)	New Zealand White x Baladi Red	5	0.5	--	--
		6	1.1	1	-6.5
		8	-2.8	2	-6.4
		10	-2.3	3	-0.9
		12	-1.6	4	1.9
Abdel-Ghany et al. (2000a)	New Zealand White x Baladi Black	6	-8.7	6-8	38.3
		8	2.8	8-10	30
		10	5.9	10-12	21.7
		12	6.7	12-14	27.8
		14	10.3	14-16	20.1
		16	7.1	--	--
Khalil and Afifi (2000)	Gabali x New Zealand White	4	2.1	--	--
		6	-2.7	--	--
		8	-0.9	--	--
		10	-3.3	--	--
		12	0.4	--	--
Abou Khadiga et al. (2008)	V line x Baladi Black	4	1.5	4-8	3.2
		8	4.4	8-12	4.6
		12	5.9	4-12	3.9
Hekil et al. (2011)	New Zealand White x Californian	4	5.3	4-6	-3.3
		6	4.1	6-8	-4.8
		8	2.8	8-10	-1.2
		10	2.7	10-12	-0.75
		12	2.9	4-12	-2.1

The first character of breed used as a sire and the next character used as a dam

2.2.3 Heterosis estimates (H^I):

The estimates of H^I for growth traits summarized from the Egyptian studies are cited in **Table 3**. These estimates showed significant direct heterosis for body weights at 4, 6 and 8 weeks of age. In this concern, **Afifi et al. (1994)** found that heterosis percentages in crossing New Zealand White with Baladi rabbits in Egypt were positive and ranged from 2.7 to 9.5% for post-weaning body weights and daily gains. **Iraqi et al. (2008)** found that the estimates of heterosis were always positive and significant, being 6.9, 3.6 and 5.4% for body weights at 4, 8 and 12 weeks and 9.7 and 6.1% for daily weight gains at intervals of 4-8 and 8-12 weeks, respectively. **Ibrahim et al. (2008)** showed that direct heterosis were positive and significant for body weights at 8 and 12 weeks of age. **Youssef et al. (2009)** found that direct heterosis were positive and ranged from 4.9 to 16.7% for body weights and 14.4 to 29.5% for daily gains. However, **Khalil (2010)** reported that direct heterosis for body weights of rabbits raised in hot countries was mainly positive and ranging from 1.3 to 14.5 %.

In non-Egyptian studies, **Ea (1999)** and **Orengo et al. (2005)** in a crossbreeding experiment including V line in Spain reported that direct and maternal heterosis for body weights at 32 and 60 days and daily weight gains between the two ages were non-significant. **Medellin and Lukefahr (2001)** stated that estimates of direct heterosis for the cross between Altex rabbits and New Zealand White were 6.6 g for weaning weight at 28 days and 1.7 g/d for average daily gain between 28–70 days ($P < 0.01$).

Table 3: Percentages of heterosis (H^I %) for body weights and daily weight gains as cited in the Egyptian literature

Reference	Breeds or strains ⁺ used in crossbreeding	Body weight		Daily weight gains	
		Age (week)	H^I %	Age interval	H^I %
Afifi et al. (1994)	New Zealand White x Baladi Red	5	2.5	--	--
		6	4	5-6	9.5
		8	5.2	6-8	7.2
		10	4.3	8-10	2
		12	3.7	10-12	0.7
Abou Khadiga et al. (2008)	V line x Baladi Black	4	10.8	4-8	7.0
		8	8.0	8-12	11.8
		12	9.1	4-12	10.0
Iraqi et al. (2008)	Sinai Gabali x V line	4	6.8	--	--
		8	3.5	4-8	3.7
		12	5.4	8-12	6.1
Youssef et al. (2009)	V line x Baladi Red	4	4.9	4-6	29.5
		6	13.0	6-8	14.4
		8	14.0	8-10	17.8
		10	16.1	10-12	18.8
		12	16.7	4-10	20.9
Hekil et al. (2011)	New Zealand White x Californian	4	9.4	--	--
		6	7.0	--	--
		8	5.6	--	--
		10	4.7	--	--
		12	4.3	--	--
Abdel-Hamid (2015)	New Zealand White× Californian	4	14.8	4-6	25.9
		6	20.2	--	--
		8	12.8	--	--
		10	9.2	10-12	11.7
		12	9.5	4-12	7.6
	New Zealand White × Rex	4	3.9	6-8	1.2
	Californian × Rex	4	12.1	10-12	5.3
		6	0.8	--	--

The first character of breed used as a sire and the next character used as a dam

Khalil et al. (2005) in crossing V line with Saudi Gabali reported significant estimates of direct heterosis for most body weights and ranging from 1.3 to 4.5% and daily weight gains from 1.3 to 5.5%. For Californian, American Chinchilla and New Zealand White breeds and nine crosses between them, **Ouyed and Brun (2008a)** found that direct heterotic effects were significant for body weights, particularly in the crosses involving the New Zealand White breed, with a magnitude ranging from 5 to 10%. **Khalil and Al-Homidan (2014)** in Saudi Arabia found that direct heterosis were significantly positive and ranging from 4.5 to 5.4 % for body weights and from 6.6 to 9.6 % for daily gains.

2.2.4 Reviewed heritabilities (h^2) estimated by the animal model for growth traits:

Reviewed heritabilities estimated by the animal model for body weights and daily weight gains in different breeds of rabbits were given in **Tables 4 and 5**. In the Egyptian studies, **Khalil and Afifi (2000)** recorded higher heritability in New Zealand White than those in Californian rabbits; the estimates for post weaning body weights ranged from 0.049 to 0.501 in New Zealand White vs. 0.07 to 0.18 in Californian and for daily weight gains from 0.14 to 0.52 in New Zealand White vs. 0.021 to 0.154 in Californian. **El-Feky et al. (2001)** showed that heritabilities for body weights at birth, 4, 8 and 16 weeks of age were 0.6, 0.85 and 0.33 in Baladi Red rabbits. **Enab et al. (2000)** reported moderate heritabilities values in New Zealand White rabbits for body weights at 4, 8 and 12 weeks of age to be 0.24, 0.31 and 0.30 whereas the estimates in Californian were 0.21,

0.20 and 0.12, respectively. **Iraqi et al. (2002)** found that heritabilities estimated by multi-trait animal model for body weight at 8 and 12 weeks of age were 0.34 and 0.30 in New Zealand White and 0.10 and 0.25 in Z line rabbits, respectively. **Youssef (2004)** reported that heritabilities of body weights at 6, 8, 10 and 12 weeks of age were 0.12 ± 0.01 , 0.16 ± 0.02 , 0.18 ± 0.04 and 0.21 ± 0.09 in New Zealand White rabbits and 0.18 ± 0.10 , 0.20 ± 0.09 , 0.23 ± 0.07 and 0.23 ± 0.07 in Baladi Red rabbits, respectively. **Iraqi (2008)** in Sinai Gabali rabbits reported that heritability estimates of body weights at 4, 8 and 12 weeks were 0.05 ± 0.059 , 0.38 ± 0.027 and 0.20 ± 0.007 , respectively. **Youssef et al. (2009)** found that heritability estimates in Baladi Black recorded slightly higher estimates followed by V line then New Zealand White and the estimates in all genetic groups were low and ranged from 0.09 to 0.21 at 4, 6, and 8 weeks of age and from 0.31 to 0.4 at 10 and 12 weeks. **Hekil et al. (2011)** reported that heritability estimates for body weights in New Zealand White rabbits at 4, 6, 10 and 12 weeks were high, being 0.58, 0.63, 0.53 and 0.43, respectively.

Table 4: Reviewed heritabilities (h^2) estimated by the animal model for post-weaning body weights at various ages in different rabbits populations

Reference and country of work	Breeds used	$h^2 \pm SE$
El-Raffa (1994), Germany	New Zealand White	
8-week weight		0.43±0.08
12-week weight		0.52 ±0.08
El-Deghadi (1996) , Egypt	New Zealand White	
8-week weight		0.14±0.05
12-week weight		0.07±0.03
Ahmed, (1997), Egypt	New Zealand White	
8-week weight		0.20 ±0.01
12-week weight		0.22±0.11
Khalil and Afifi (2000), Egypt	New Zealand White	
5-week weight		0.35±0.120
6-week weight		0.50±0.165
8-week weight		0.14±0.049
10-week weight		0.05±0.018
12-week weight		0.07±0.026
Khalil and Afifi (2000), Egypt	Californian	
5-week weight		0.22±0.07
8-week weight		0.09±0.014
10-week weight		0.07±0.91
12-week weight		0.26±0.11
El-Feky et al. (2001), Egypt	Baladi Red	0.33±0.23
Enab et al. (2000), Egypt	New Zealand White	
4-week weight		0.24
8-week weight		0.31
12-week weight		0.30
Enab et al. (2000), Egypt	Californian	
4-week weight		0.21
8-week weight		0.20
12-week weight		0.12
Iraqi et al. (2002), Egypt	New Zealand White	
8-week weight		0.34
12-week weight		0.30
García and Baselga (2002), Spain	V line	
4-week weight		0.22±0.009
9-week weight		0.30±0.013

SE=Standard error

Table 4: Cont.

Reference and country of work	Breeds used	$h^2 \pm SE$
Khalil et al. (2005), Saudi Arabia	V line x Saudi Gabali	
4-week weight		0.24 \pm 0.073
6-week weight		0.10 \pm 0.025
8-week weight		0.08 \pm 0.019
10-week weight		0.19 \pm 0.020
12-week weight		0.13 \pm 0.023
Akanno and Ibe (2005), Nigeria	New Zealand White and Dutch	
6-week weight		0.43 \pm 0.35
9-week weight		0.11 \pm 0.22
12-week weight		0.36 \pm 0.35
Iraqi (2008), Egypt	Sinai Gabali	
4-week weight		0.05 \pm 0.059
8-week weight		0.38 \pm 0.027
12-week weight		0.20 \pm 0.007
Youssef et al. (2009), Egypt	APRI line and Zealand White	
4-week weight		0.24 \pm 0.073
6-week weight		0.09 \pm 0.025
8-week weight		0.08 \pm 0.019
10-week weight		0.19 \pm 0.020
12-week weight		0.13 \pm 0.023
Hekil et al. (2011), Egypt	New Zealand White	
8-week weight		0.15 \pm 0.150
12-week weight		0.43 \pm 0.532
Hekil et al. (2011), Egypt	Californian	
4-week weight		0.61 \pm 0.460
6-week weight		0.09 \pm 0.112
10-week weight		0.11 \pm 0.130
12-week weight		0.23 \pm 0.225
Dige et al. (2012), India	New Zealand White	
15th day body weight		0.48 \pm 0.03
30th day body weight		0.44 \pm 0.02
90th day body weight		0.33 \pm 0.03
180th day body weight		0.23 \pm 0.03
Khalil and Al-Homidan (2014), Saudi Arabia	Saudi Gabali	
4-week weight		0.24 \pm 0.073
6-week weight		0.19 \pm 0.025
8-week weight		0.27 \pm 0.019
10-week weight		0.19 \pm 0.020
12-week weight		0.27 \pm 0.023

SE=Standard error

Table 5: Reviewed heritabilities (h^2) estimated by the animal model for daily weight gains (DG) at various age intervals in different rabbits populations

Reference and country of work	Breeds used	$h^2 \pm SE$
El-Raffa, (1994), Egypt DG8-12	New Zealand White	0.54 \pm 0.09
Khalil and Afifi (2000), Egypt DG4-6	New Zealand White	0.52 \pm 0.170
DG6-8		0.18 \pm 0.065
DG8-10		0.14 \pm 0.053
DG10-12		0.21 \pm 0.074
Khalil and Afifi (2000), Egypt DG4-6	Californian	0.11 \pm 0.065
DG6-8		0.07 \pm 0.034
DG8-10		0.07 \pm 0.032
DG10-12		0.18 \pm 0.074
Khalil et al. (2005), Saudi Arabia DG4-6	V line x Saudi Gabali	0.09 \pm 0.034
DG6-8		0.04 \pm 0.010
DG8-10		0.02 \pm 0.011
DG10-12		0.02 \pm 0.011
DG4-10		0.09 \pm 0.030
Iraqi (2008), Egypt DG4-8	Sinai Gabali	0.23 \pm 0.141
DG8-12		0.19 \pm 0.193
Youssef et al. (2009), Egypt DG6-8	APRI line	0.04 \pm 0.010
DG8-10		0.02 \pm 0.011
DG10-12		0.02 \pm 0.008
DG4-10		0.09 \pm 0.030
DG4-12		0.10 \pm 0.040
Hekil et al. (2011), Egypt DG 6–8	Zealand White	0.35 \pm 0.271
DG 8–10		0.44 \pm 0.202
DG 10–12		0.19 \pm 0.118
Hekil et al. (2011), Egypt DG6–8	Californian	0.36 \pm 0.184
DG8–10		0.49 \pm 0.226
DG10–12		0.48 \pm 0.222

SE= Standard error

Table 5: Cont.

Reference and country of work	Breeds used	$h^2 \pm SE$
Khalil and Al-Homidan (2014), Saudi Arabia	Saudi Gabali	
DG4-6		0.28±0.034
DG6-8		0.34±0.010
DG8-10		0.28±0.011
DG10-12		0.24±0.008
DG4-10		0.19±0.030
DG10-12		0.18±0.040

SE= Standard error

In non-Egyptian studies, the heritabilities for weaning weight (4 weeks) and slaughter weight (63 days) in the Spanish V line were 0.224 ± 0.009 and 0.302 ± 0.013 , respectively (**García and Baselga, 2002**). **Akanno and Ibe (2005)** in Nigeria found that heritability estimates for body weights at 6, 9 and 12 weeks of age in New Zealand White rabbits were 0.43 ± 0.35 , 0.11 ± 0.22 and 0.36 ± 0.35 , respectively. **Niranjan et al. (2010)** found that the estimates of heritability for weights at 6, 12, 18 and 24 weeks were 0.25 ± 0.05 , 0.17 ± 0.05 , 0.21 ± 0.06 and 0.12 ± 0.05 in Angora rabbits, respectively. Heritabilities for growth traits were mostly moderate and ranged from 0.19 to 0.27 for body weights and from 0.18 to 0.34 for daily weight gains in synthetic line of Saudi-1 and Saudi 2 rabbits (**Khalil and Al-Homidan, 2014**).

2.3 Crossbreeding effects on semen traits:

2.3.1 Genetic groups comparisons for semen traits:

The means of semen traits reviewed for different genetic groups of rabbits are given in **Table 6**.

Table 6: Reviewed means of semen traits in different genetic groups of rabbits

Reference	Breeds used and genetic groups	EV (ml)	pH	MS (%)	LS (%)	AS (%)	SC X10 ⁶
Khalil et al. (2002)	V line	0.62	7.1	56.7	90.8	10.7	385
	Gabali	0.56	5.4	50.6	89.1	8.7	512
	½ V line ½ Gabali	0.59	6.8	62.7	91.9	6.8	--
	½ Gabali ½ V line	0.66	6.86	79.3	93.8	6.1	--
	¾ V line ¼ Gabali	0.60	6.9	79.3	89.1	8.5	--
Brun et al. (2006)	L line	0.6	6.94	76.3	--	--	634
	H line	0.46	6.93	75.8	--	--	738
García-Tomás et al. (2006)	C line	1.21	7.8	--	--	14.8	251
	R line	1.09	7.6	--	--	14.3	249
	C line x R-line	1.14	7.5	--	--	13.8	306
	R line x C-line	1.31	7.6	--	--	11.4	208
Khalil et al. (2007)	V line x Gabali Saudi	0.63	7.5	65.7	91.9	14.6	434
Lavara et al. (2008)	R line	0.62	--	--	--	17.0	193
El-Azim and El-Kamash (2011)	Californian	0.74	7.5	63.1	82.2	14.1	340
	New Zealand White	0.8	7.42	68.2	86.1	12.5	341
	Sinai	0.65	7.33	63.1	80.1	16.8	353
	Baladi	0.65	7.35	63.2	81.4	16.7	357
Iraqi et al. (2012)	Gabali	0.60	7.74	46.9	80.6	11.7	405
	V line	0.60	7.79	--	82.3	--	474
	Moshtohor line	0.72	--	48.6	81.9	12.9	456
Abou Khadiga et al. (2012)	APRI line	1.36	6.3	68.3	80.1	12.2	--
El-Tarabany et al. (2015)	New Zealand White	0.52	7.5	--	94.1	3.9	737
	Flander	0.45	7.6	--	94.6	5.6	660
	Rex	0.57	7.8		92	4.96	511
Brun et al. (2016)	INRA1001 line	0.5	6.9	70.3	--	--	663
Anous et al. (2017)	New Zealand White	0.64	6.9	85.5	93.1	8.3	469
	Californian	0.79	6.9	87.5	94.6	5.8	480
	Rex	0.79	6.7	93.4	96.3	3.7	415
	Gabali	0.55	6	88.9	94.2	7.4	191

EV= Ejaculate volume, pH= pH of semen, MS= Motility of sperms (%), LS= Live sperms (%), AS= Abnormal sperms (%) and SC= Sperms concentrationX10⁶/ml.

2.3.1.1 Ejaculate volume (ml):

Balbaa (1981) reported a significant increase in semen volume of New Zealand White bucks (0.74 ml) compared to the Egyptian Baladi bucks (0.44 ml). **Mehrez et al. (1981)** specified the presence of a non-significant higher ejaculate volume of Bouscat bucks (0.71 ml) than that of Baladi bucks (0.39 ml). **Dubiel et al. (1985)** showed significant differences in semen ejaculate volume between bucks of Black Tan (0.68 ml), New Zealand White (0.97 ml), New Zealand Red (0.83 ml) and German Pied Giant (1.51 ml). **Abd-El-Hakeam et al. (1992)** reported a significant divergence in the semen ejaculate volume between New Zealand White bucks (0.58 ml) and Californian bucks (0.47ml). **Al-sobayil and Khalil (2002)** found that the differences in ejaculate volume between V line and Saudi Gabali bucks were significant ($P<0.05$). **Khalil et al. (2002)** found that the ejaculate volume of V line bucks (0.62 ml) was higher than that of Gabali bucks (0.56 ml). **Brun et al. (2006)** reported a significant difference in semen ejaculate volume between bucks of L line (0.6 ml) and R line (0.46 ml). **García-Tomás et al. (2006)** found that the differences in semen ejaculate volume between C line bucks (1.21 ml) and R line bucks (1.09 ml) were relevant ($P<0.05$). **El-Azim and El-Kamash (2011)** reported that Californian bucks (0.74 ml) had higher ejaculate volume compared with bucks of New Zealand White (0.8 ml), Sinai Gabali (0.65 ml) and Baladi (0.65 ml). **Iraqi et al. (2012)** reported significant differences in semen ejaculate volume between Gabali, V line and Moshtohor line bucks, being 0.60, 0.66 and 0.72 ml, respectively. **El-Tarabany et al. (2015)**

found that the ejaculate volume of Rex bucks (0.75 ml) was higher than that of New Zealand White (0.52 ml) and Flander (0.45 ml). **Anous et al. (2017)** reported significant ($P < 0.05$) differences between bucks of New Zealand White, Californian, Rex and Gabali rabbits, being 0.64, 0.7, 0.79 and 0.55 ml, respectively.

2.3.1.2 Semen pH:

The seminal *pH* of New Zealand White breed was significantly ($P < 0.01$) higher than that of the California breed, however, the values of both breeds were above 7.5 (**Amin et al., 1987**). The semen *pH* of some breed groups diverge from 6.8 to 8.4 and this is a good index for evaluated semen quality (**Alvariño, 2000**). **Khalil et al. (2002)** reported that the difference between V line and Gabali rabbits (7.12 and 5.47) was significant for semen *pH*. **García-Tomás et al. (2006)** reported significant difference in semen *pH* ($P < 0.05$) between bucks of C line (7.8) and R line (7.6) rabbits. **El-Azim and El-Kamash (2011)** stated that the *pH* of semen showed significant differences between bucks of Californian (7.5), New Zealand White (7.42), Sinai (7.33) and Baladi (7.35). The *pH* of semen in Rex bucks (7.87) was higher than that of Flander bucks (7.65) and New Zealand White bucks (7.56) (**El-Tarabany et al., 2015**). Recently, **Anous et al. (2017)** found that the *pH* of semen showed significant ($P < 0.05$) differences between New Zealand White (6.96), Californian (6.90), Rex (6.78) and Gabali (6.8).

2.3.1.3 Sperms motility (%):

Dubiel et al. (1985) showed significant differences in sperms motility in semen between Black Tan (54 %), New Zealand White (66%), New Zealand Red (49 %) and German Pied Giant (71 %). High sperms motility in New Zealand White rabbits was recorded to be 78.5% by **Hemid and Tharwat (1995)**. **Khalil (1999)** reported that sperms mortality in Gabali bucks (50%) was lower than that in New Zealand White (55%), i.e. sperm motility differs between the diverse breeds of rabbits. **Khalil et al. (2002) and Al-sobayil and Khalil (2002)** showed significant differences in sperms motility between bucks of V line (56.7%) and Gabali (50.6%). **Brun et al. (2006)** found that the percentage of sperm motility in H line (75.8%) was lower than that in L line (76.3%). **El-Azim and El-Kamash (2011)** found that Sperms mortality in bucks of Sinai (63.11%) and Baladi (63.21%) was lower than that in New Zealand White bucks (68.21%). The sperms motility showed significant differences between bucks of Gabali (46.9%), V line (48.6%), and Moshtohor line (54.8%) (**Iraqi et al., 2012**). **Anous et al. (2017)** specified the presence of significant differences in sperm motility between bucks of New Zealand White (85.5%), Californian (87.5%), Rex (93.4%) and Gabali (88.9%).

2.3.1.4 Live and dead sperms per ejaculate (%):

Khalil et al., (2002) found that the percentage of live sperms in semen of Gabali (89.1%) was lower than that in V line (90.8%). **El-Azim and El-Kamash (2011)** reported significant divergences in live sperms between New Zealand White bucks (86.1%) and Baladi

bucks (81.4%). **Iraqi et al. (2012)** showed that the percentage of live sperms was lesser in semen of Gabali (80.6%) than that in V line (82.3%) and Moshtohor line (81.9%). **El-Tarabany et al. (2015)** reported that the percentages of live sperms in semen of Rex (96.3%) were higher than that of New Zealand White (94.6%), California (94.6%), and Gabali (94.2%). Recently, **Anous et al. (2017)** recorded the percentages of live sperms to be 93.1% in semen of New Zealand White bucks, 94.6% in Californian bucks, 96.3% in Rex bucks and 94.2% in Gabali bucks.

2.3.1.5 Sperms abnormalities per ejaculate (%):

The sperms abnormality was recorded to be 25.9% in semen of Egyptian Baladi and 13.9% in Rex bucks (**El-Sheikh, 1991**). **Khalil et al. (2002)** specified the presence of significant differences in abnormal sperms between V line (10.7%) and Gabali (8.7%). **Seleem (2005)** specified the presence of significant differences in sperm abnormalities between Flanders (17.7%) and New Zealand White (20.5%) and their crosses (25.4%). **García-Tomás et al. (2006)** found that the percentage of abnormal sperms in bucks C-line (14.8%) was higher than that of R line (14.3%), C line x R line (13.8%) and R line x C line (11.4%). **Iraqi et al. (2012)** recorded sperms abnormality to be 11.7% in Egyptian Gabali and 12.9% in Moshtohor line bucks. **Anous et al. (2017)** found that the percentage of abnormal sperms in semen of Rex (3.7%) was lower than that of New Zealand White (8.3%), Gabali (7.4%), and California (5.8%).

2.3.1.6 Sperms cell concentration ($\times 10^6$):

Dubiel et al. (1985) stated that the sperms cell concentration in semen showed significant differences between Black Tan ($397.6 \times 10^6/ml$), New Zealand White ($309.6 \times 10^6/ml$), New Zealand Red ($221.7 \times 10^6/ml$) and German Pied Giant ($502.5 \times 10^6/ml$). **Khalil (1997)** reported that the sperms cell concentration of Gabali ($362 \times 10^6/ml$) was higher than that of New Zealand White ($329 \times 10^6/ml$), Californian ($314 \times 10^6/ml$), Giza White ($282 \times 10^6/ml$) and Baladi ($330 \times 10^6/ml$). **Alvariño (2000)** reported that sperms concentration ranged from 50×10^6 to 500×10^6 per *ml*, while the estimates of **Brun et al. (2002)** and **Nizza et al. (2003)** ranged from $427 \times 10^6/ml$ to $574 \times 10^6/ml$ in all genetic groups. **Brun et al. (2006)** reported that the sperm cell concentration evidenced significant diversities among L line and H line ($634 \times 10^6/ml$ and $738 \times 10^6/ml$, respectively). The sperms cell concentration showed significant differences between Californian ($340 \times 10^6/ml$), New Zealand White ($341 \times 10^6/ml$), Sinai ($353 \times 10^6/ml$) and Baladi ($357 \times 10^6/ml$) as stated by **El-Azim and El-Kamash (2011)**. **Iraqi et al. (2012)** reported that the sperms cell concentration evidenced significant diversities among bucks of Gabali, V line and Moshtohor line ($405 \times 10^6/ml$, $474 \times 10^6/ml$ and $456 \times 10^6/ml$, respectively). **El-Tarabany et al. (2015)** found that the sperms cell concentration in semen of New Zealand White ($737 \times 10^6/ml$) was higher than that of Flander ($660 \times 10^6/ml$) and Rex ($551 \times 10^6/ml$). **Anous et al. (2017)** found that the estimates of the sperms cell concentration ranged from $191 \times 10^6/ml$ to $480 \times 10^6/ml$ in New Zealand White, Californian, Rex and Gabali breeds studied.

2.3.2 Direct additive genetic effects (G^I):

The estimates of G^I for semen traits are summarized from literature and cited in **Table 7. García-Tomás et al. (2006)** when crossing C line with R line rabbits found that the estimates of G^I were significantly in favor of C line by 32.8% for concentration of sperms. **Khalil et al. (2007)** recorded that the estimates of G^I were significantly in favour of Saudi Gabali rabbits by 14.2% for dead sperms, 12.4% for concentration of sperms and 12% for abnormal sperms relative to V line.

Table 7: Percentages of direct additive genetic effects (G^I %) for semen traits as cited in literature

Reference and country of work	Breeds or strains ⁺ used	Semen trait	G^I %
García-Tomás et al. (2006), Spain	C line x R line	pH of semen	0.8 ^{NS}
		Ejaculate volume	-4.0 ^{NS}
		Sperms mass motility	-0.4 ^{NS}
		Individual motility	-6.8 ^{NS}
		Sperms concentration	32.8 ^{**}
Khalil et al. (2007), Saudi Arabia	V line x Saudi Gabali	Ejaculate volume	8.2 ^{NS}
		pH of semen	2.7 ^{NS}
		Sperms concentration	-12.4 [*]
		Sperms motility	-6.3 ^{NS}
		Abnormal sperms	12.0 [*]
		Dead sperms	14.2 [*]

The first letter of breed used as a sire and the next letter used as a dam; NS= non-significant, *= $P \leq 0.05$ and **= $P \leq 0.01$

2.3.3 Maternal additive effect (G^M):

The estimates of G^M for semen traits are reviewed and cited in **Table 8. García-Tomás et al. (2006)** with two sire lines (C and R) observed differences between the two lines in maternal genetic effects and these differences were significantly important for

ejaculate volume (14%) and in favor of C line and significantly in favor of R line for concentration of sperms (40%), number of spermatozoa per ejaculate (23%), mass and individual motility and percentage of spermatozoa with presence of morphological abnormalities of neck-piece. Also, **Khalil et al. (2007)** showed that the differences in the maternal additive effects for volume of ejaculate (11.7%) and libido score (6.9%) were significantly in favor of V line and significantly in favor of Saudi rabbits for concentration of sperms (10.0%), percentage of abnormal sperms (13.2%), percentage of dead sperms (18.6%) and percentage of sperms motility (10.8%).

Table 8: Percentages of maternal additive effects (G^M %) for semen traits as cited in literature

Reference and country of work	Breed or strain ⁺ used	Semen trait	G^M %
García-Tomás et al. (2006), Spain	C line x R line	pH of semen	1.8 ^{**}
		Ejaculate volume	14.0 [*]
		Sperms mass motility	-8.5 [*]
		Individual motility	-16.4 [*]
		Sperms concentration	40 ^{**}
		Number of sperms per ejaculate	23 ^{**}
Khalil et al. (2007), Saudi Arabia	V line x Saudi Gabali	Ejaculate volume	11.7 [*]
		pH of semen	4.0 ^{NS}
		Sperms concentration	-10.0 [*]
		Sperms motility	-10.8 [*]
		Abnormal sperms	13.2 [*]
		Dead sperms	18.6 [*]

The first letter of breed used as a sire and the next letter used as a dam; NS= non-significant, ^{*}= $P \leq 0.05$ and ^{**}= $P \leq 0.01$.

2.3.4 Heterosis estimates (H^I):

The estimates of H^I for semen traits are reviewed from literature and cited in **Table 9**. In general, crossbreeding among breeds raised in hot climates was associated with heterotic effects in semen characteristics as stated by **Abo El-ezz et al. (1985)** and **El-Tarabany et al. (2015)** in Egypt. Recently, **El-Tarabany et al. (2015)** found that there were positive estimates of direct heterosis for ejaculate volume, sperms mass motility, individual motility and sperms cell concentration in three crossbreeding experiments between New Zealand White and Flander rabbits, New Zealand White x Rex, New Zealand White x Flander and Rex x Flander. **Al-sobayil and Khalil (2002)** in Saudi Arabia estimated moderate direct heterosis to be 21.5% for percentages of abnormal sperms ($P < 0.05$) and 20.3% for dead sperms ($P < 0.05$). In addition, **Khalil et al. (2007)** reported that crossbred bucks were associated with syndicate heterotic effects in ejaculate volume (11.6%; $P < 0.05$), sperms concentration (10.5%; $P < 0.05$), percentages of motile sperms (9.8%; $P < 0.05$) along with a reduction in percentages of abnormal sperms (-10.8%) and dead sperms (23.5%; $P < 0.05$). **Brun et al. (2002)** in France reported high estimates of heterosis to be 6.8% in mass motility and 4.1% in percentage of sperms motility along with high values in sperms concentration (37.5%), total number of spermatozoa per ejaculate (37.6%) and number of sperms motility per ejaculate (42.3%). Also, **García-Tomás et al. (2006)** in Spain reported high variability in the estimates of direct heterosis for several semen

characteristics, being practically negligible for sperms normality (about 2%), but very high for the percentage of spermatozoa (57%).

Table 9: Percentages of heterosis (H^1 %) for semen traits in different rabbit populations as cited in literature

Reference and country of work	Breed or strain ⁺ Used	Semen trait	H ¹ %
Brun et al. (2002), France	INRA160 line x INRA2066 line	Sperms mass motility	6.2 [*]
		Sperms motility	4.1 [*]
		Sperms concentration	37.5 [*]
García-Tomás et al. (2006), Spain	C line x R line	pH of semen	-1.3 ^{NS}
		Ejaculate volume	6.7 ^{NS}
		Sperms mass motility	4.1 ^{NS}
		Individual motility	8.2 ^{NS}
		Sperms concentration	2.1 ^{NS}
Khalil et al. (2007), Saudi Arabia	V line x Saudi Gabali	Ejaculate volume	10.6 [*]
		pH of semen	1.6 ^{NS}
		Sperms concentration	13.5 [*]
		Sperms motility	10.5 [*]
		Abnormal sperms	-21.5 [*]
		Dead sperms	-20.3 [*]
El-Tarabany et al. (2015), Egypt	New Zealand White x Rex	Ejaculate volume	-13.4 ^{**}
		pH of semen	-0.5 ^{NS}
		Sperms mass motility	0.2 ^{NS}
		Individual motility	-1.6 ^{NS}
		Sperms concentration	-16.1 ^{**}
		Live sperms	-0.03 ^{NS}
El-Tarabany et al. (2015), Egypt	New Zealand White x Flander	Ejaculate volume	17.4 ^{**}
		pH of semen	-1.8 ^{NS}
		Sperms mass motility	0.5 ^{NS}
		Individual motility	2.4 [*]
		Sperms concentration	13.5 [*]
		Live sperms	1.8 [*]
El-Tarabany et al. (2015), Egypt	Rex x Flander	Ejaculate volume	-5.8 [*]
		pH of semen	-2.7 [*]
		Sperms mass motility	6.2 [*]
		Individual motility	0.16 ^{NS}
		Sperms concentration	19.8 [*]
		Live sperms	1.1 ^{NS}

The first character of breed used as a sire and the next used as a dam; NS= non-significant, *= $P \leq 0.05$ and **= $P \leq 0.0$.

Review Of Literature

2.3.5 Reviewed heritabilities (h^2) estimated by the animal model for semen traits:

The reviewed estimates of heritability computed by the animal model for semen traits in different breeds of rabbits were mostly low as shown in **Table 10**.

Table 10: Reviewed heritabilities (h^2) estimated by the animal model for semen traits in different populations of rabbits

Reference and Country of research	Breeds used	Trait	$h^2 \pm SE$
Khalil et al. (2007), Saudi Arabia	Saudi line	Ejaculate volume	0.13 \pm 0.020
		<i>pH</i> of semen	0.12 \pm 0.028
		Sperms concentration	0.12 \pm 0.026
		Sperms motility	0.18 \pm 0.025
		Abnormal sperms	0.16 \pm 0.023
		Dead sperms	0.17 \pm 0.021
Lavara et al. (2008), Spain	R Line	Ejaculate volume	0.091 \pm 0.048
		Sperms concentration	0.053 \pm 0.043
		Total number of spermatozoa per ejaculate	0.097 \pm 0.034
Lavara-Culebras et al. (2010), Spain	R Line	Ejaculate volume	0.13 \pm 0.05
		Sperms concentration	0.08 \pm 0.04
		Sperms production in $\times 10^6$	0.07 \pm 0.03
Lavara et al. (2011), Spain	(R Line) ² Interse	Ejaculate volume	0.13
		Sperms concentration	0.08
		Number of sperms per ejaculate	0.07
Brun et al. (2016), France	INRA1001	<i>pH</i> of semen	0.06 \pm 0.02
		Ejaculate volume	0.06 \pm 0.03
		Mass motility	0.05 \pm 0.03
		Sperms concentration	0.10 \pm 0.03
		Sperms motility	0.18 \pm 0.04

SE=Standard error.

Khalil et al. (2007) in Saudi Arabia reported low estimates of heritability to be 0.13, 0.12, 0.12, 0.18, 0.16 and 0.17 for ejaculate volume, *pH* of semen, sperms concentration, sperms motility,

abnormal sperms, and dead sperms, respectively. **Lavara et al. (2007)** and **Lavara et al. (2013)** reported also low estimates of heritability to be from 0.07 to 0.13 for individual sperm motility, sperms mass motility, ejaculate volume, sperms concentration and sperms production per ejaculate. Recently, **Brun et al. (2016)** in France reported also low estimates of heritability to be from 0.05 to 0.18 for semen traits in INRA1001 rabbits.

2.4 Genotyping techniques:

2.4.1 Detection of DNA polymorphism (RAPD, AFLP and RFLP):

For animal genotyping, DNA polymorphisms are detected by varieties of techniques, the most common being randomly amplified polymorphic DNA (RAPD) (**Nagy et al., 2010; Jawasreh et al., 2011; Qasim et al., 2011**), single stranded conformation polymorphisms (SSCP) (**Bastos et al., 2001**), amplified fragment length polymorphisms (AFLP) and restriction fragment length polymorphisms (RFLP) (**Abdel-rahman et al., 2010**). These polymorphic procedures have been used for several purposes like genetic identification of inbred strains of the rabbit (*Oryctolagus cuniculus*), quantitative traits loci (**Xiao et al., 2009**), variable number of tandem repeats (VNTR), microsatellites as short tandem repeats (STR), diversity analysis (**Zenger et al., 2003**) and single nucleotide polymorphisms (SNP) (**Pariset et al., 2006**).

For the most common technique, the PCR-RFLP consists of two main steps: first, amplifying DNA using the standard PCR (i.e. amplification), second, digesting PCR product using restriction

enzymes (i.e. digestion). **Hailu and Getu (2015)** stated that the most important advantages of the PCR-RFLP technique include: 1) It was widely applied for the analysis of genetically determined diseases in poultry, 2) Inexpensiveness and lack of requirements for advanced instruments, 3) It has easy design and can be accomplished using public available programs, 4) RFLP is labor-intensive and time-consuming, 5) It can be used to check out only specific mutations at enzyme cut sites, 6) It is relatively low for detecting the polymorphism, 7) It has high reliability, because it is generated from specific sites via known restriction enzymes and the results are constant over time and location, and 8) It can be used to detect the oncogenes in gene mapping and phylogenetic analysis and for the study of association relations of candidate genes with performance indicators. The disadvantages of the PCR-RFLP method as stated by **Rasmussen (2012)** include: 1) It requires specific endonucleases in identifying the exact variation, and 2) It requires several SNP in affecting the same restriction enzyme recognition site.

2.4.2 Molecular markers:

Genetic markers provide information as bioinformatics indicators about polymorphism in allelic genotype at a given locus. The availability of molecular markers in farm animals allows the detailed analyses and evaluation of genetic diversity and furthermore the detection of genes influencing economically important traits. Molecular markers should not be considered as normal genes as they usually do not have any biological effect. They are identifiable DNA sequences, found in specific locations of the genome, and transmitted

by inheritance from one generation to the next, allowing the assessment of genetic variability among genotypes at DNA level.

Among all types of molecular markers, the microsatellites are used as the most widely markers for the analysis of genetic diversity and population structure of livestock (**Maudet et al., 2002**). As stated by **Erhardt and Weimann (2007)**, the majority of molecular markers used are microsatellite markers, STR (short tandem repeats) and SNP (single nucleated polymorphism). At present, DNA molecular marker techniques are widely applied in the fields of germplasm identification, phylogenetic analysis, and genetic structural analysis (**Yang et al., 2013**). As stated by **Marko et al. (1999)**, SNP has the following advantages over the other types of genetic markers: 1) It has high level of polymorphism, 2) It has distribution throughout the genome, 3) It has the presence within coding regions, 4) It has introns and regions that flank the genes, 5) It is simple and unambiguous assay technique, 6) It has stable Mendelian inheritance, and 7) It has low levels of spontaneous mutation. In comparison with the highly polymorphic microsatellite markers, SNP has the following advantages (1) It is less informative due to its biallelic nature, (2) It has significant advantages over microsatellite markers as a basis for high-resolution whole genome allelotyping because of their abundance, even spacing, and stability across the genome, and (3) It is used to identify the paternal and maternal alleles of a given gene based on polymorphisms. As stated by **Seidel (2009)**, the genomic selection using the SNP markers is a powerful new tool because: 1) SNP can be detected by a number of

methods such as PCR-RFLP, 2) SNP is relatively new technology using DNA chips that can be used for large scale screening of numerous samples in a minimal amount of time (**Fontanesi et al., 2008**), 3) SNP is the most recent contribution to study DNA sequence variation, and 4) SNP represents the most innovative molecular marker in genotyping studies. However, recent advances in high-throughput DNA sequencing, computer software and bioinformatics have facilitated the identification of SNP as molecular markers.

The microsatellite has been used to develop the markers from genes and they have been referred as genic molecular markers (GMMs) or functional markers (FMs). They compared the SNP results with the analysis using microsatellites and concluded that: 1) microsatellites provide high clustering success due to high polymorphic nature, 2) SNP provides broader genome coverage and reliable estimates of genetic relatedness in the genome, and 3) SNP considered to be an efficient and cost-effective genetic tool.

2.5 Candidate genes and their association with growth traits:

Research on numerous candidate genes have been conducted and confirmed the fact that there are polymorphic associations between candidate genes and growth traits in different genetic groups of rabbits as shown in **Table 11**.

Hull and Harvey (2000) recorded that growth hormone gene (*GH*) is not classically considered as a reproductive hormone gene; although it has some functions, like: (1) It has great roles in reproductive function and secretion and action of LH and FSH, (2) It is required for sexual differentiation and pubertal maturation, (3) It

participates in gonadal steroid genesis, gametogenesis and ovulation, and (4) It required for fetal nutrition and growth during pregnancy and for mammary development and lactation. **Hristova et al. (2018)** analyzed 86 rabbits (*Oryctolagus cuniculus*) and reported that the single nucleotide polymorphisms for the studied *GH* locus corresponding to three genotypes were CC, CT and TT. **Abdel-Kafy et al. (2016)** in 118 ♀ and 84 ♂ evaluated that the effects of the growth hormone gene polymorphisms (*GH*) on reproduction and growth traits in APRI rabbit and DNA was extracted from rabbit blood samples to be used in PCR amplification. The c.-78C>T SNP was genotyped by PCR-RFLP using *Bsh1236I* (*BstUI*) digestion restriction enzyme and the associations analyses between the *GH* C>T SNP and body weights and reproductive traits were tested.

Abdel-Kafy et al. (2016) estimated the association between the *GH* C>T SNP with body weight, growth and reproductive traits in rabbit populations and reported that: (1) Heterozygote genotype (T/C) was significantly associated with heavy weight of rabbits at 8 weeks and daily gain through 5-8 week interval compared to homozygous TT and CC genotypes ($P < 0.05$), (2) The polymorphism of growth hormone gene (*GH*) in rabbits may has over-dominance at the locus c.-78C>T, and (3) Positive effects of the heterozygous genotype were recorded compared to both homozygous genotypes on body weights and body gains, i.e. the heterozygous genotype in c.-78C>T of *GH* polymorphisms could be used as a favorable genotype in rabbits and

may be used in assisted marker in selection programs (MAS) to improve growth performance.

Table 11: Reviewed candidate genes associated with economic traits in different genetic groups of rabbits

Chromosome	Candidate gene	Associated trait	Breed group used	Reference and country of work
1	Progesterone receptor (<i>PGR</i>)	Litter size	H and L lines	Peiró et al. (2008), Spain.
		Body weight	V line, Sinai Gabali	El-Aksher et al. (2016), Egypt
3	Fibroblast growth factor (<i>FGF</i>)	Body weight at 23-days, 28-days and 33-days of age	V line, Alexandria	El-Sabroun and Aggag (2017), Egypt
4	Insulin-like Growth Factor 1 (<i>IGF-1</i>)	Body weight at 23-days, 28-days and 33-days of age	V line, Alexandria	El-Sabroun and Aggag (2017), Egypt
	Insulin-like growth factor 2 (<i>IGF2</i>)	Body weight at 70-days of age	Genetic group different Rabbit	Fontanesi et al. (2012), Italy
7	Myostatin (<i>MSTN</i>)	Meat production	Belgian Hare, Burgundy Fawn, Checkered Giant, Giant Grey	Fontanesi et al. (2008), Italy
		Growth traits Carcass traits	Z ₂ line, Z ₄ line, Z ₂ ×Z ₄ cross	Lu et al. (2011), China
		Body weight at 84- days of age	Ira, Champagne, Tianfu black	Peng et al. (2013), China
	Myostatin (<i>MSTN</i>)	Body weight at 23-days, 28-days and 33-days of age	V line, Alexandria	El-Sabroun and Aggag (2017), Egypt

Table 11: Cont.

Chromosome	Candidate gene	Associated trait	Breed group used	Reference and country of work
	Leptin (<i>LEP</i>)	Carcass trait, Meat quality	Zealand White, Belgian Giant, Giant Grey	Migdal et al. (2018), Poland
9	Melanocortin 4 receptor (<i>MC4R</i>)	Body weight at 23-days, 28-days and 33-days of age	V line, Alexandria	El-Sabroun and Aggag (2017), Egypt
10	phosphoglycerate mutase (<i>PGAM2</i>)	Body weight at 84-days of age	Tianfu black, Ira, Champagne	Wu et al. (2015), China
11	Growth hormone receptor (<i>GHR</i>)	Body weight at 28-days, 35-days and 70-days of age	Tianfu black, Ira, Champagne	Zhang et al. (2012), China
		Growth traits	New Zealand White, V line, Californian, Alexandria	Sahwan et al. (2014), Egypt
		Body weight at 23-days, 28-days and 33-days of age	V line, Alexandria	El-Sabroun and Aggag (2017), Egypt
	Calpastatin (<i>CAST</i>)	Meat quality	Champagne, Tianfu Black	Wang et al. (2016), China
12	Basic fibroblast growth factor (<i>BFGF</i>)	Body weight at 3, 4 and 10 weeks of age	knee rabbit joints	Inoue et al. (2006), Japan.
14	<i>POU1F1</i> gene	Meat quality	Hyla, Champagne, Tianfu Black	Wang et al. (2015), China
15	Fibroblast growth factor 5 (<i>FGF-5</i>)	Body weight	Local Egyptian	Othman et al. (2015), Egypt

Table 11: Cont.

Chromosome	Candidate gene	Associated trait	Breed group used	Reference and country of work
18	Phosphoglucose mutase (<i>PGAM</i>)	Body weight at 23-days, 28-days and 33-days of age	V line, Alexandria	El-Sabrout and Aggag (2017), Egypt
19	Growth hormone (<i>GH</i>)	Meat production	Belgian Hare, Burgundy Fawn, Checkered Giant, Giant Grey	Fontanesi et al. (2008), Italy
		Body weight at 8 weeks of age	APRI Line	Abdel-Kafy et al. (2016), Egypt
		Body weight at 23-days, 28-days and 33-days of age	V line, Alexandria	El-Sabrout and Aggag (2017), Egypt

There were significant associations between the genotypes SNP and body weights. In this concept, the growth hormone gene (*GH*) located on chromosome 19, growth hormone receptor gene (*GHR*) located on chromosome 11, progesterone receptor gene (*PGR*) located on chromosome 1, fibroblast growth factor gene (*FGF*) located on chromosome 3, insulin-like growth factor 1 and 2 genes (*IGF1* and *IGF2*) located on chromosome 4, myostatin gene (*MSTN*) located on chromosome 7 and melanocortin 4 receptor gene (*MC4R*) located on chromosome 9 have shown significant associations with body weights in rabbits (Inoue et al., 2006; Fontanesi et al., 2008; Lu et al., 2011; Zhang et al., 2012; Fontanesi et al., 2012; Peng et al., 2013; Sahwan et al., 2014; Wu et al., 2015; Othman et al., 2015; El-Aksher et al., 2016; El-Sabrout and Aggag, 2017; Migdal et al., 2018).

2.6 Candidate genes and their associations with semen traits:

Kmieć et al. (2007) showed that genotype BB of the *GH* gene produced larger ejaculates volume and higher sperms percentage as compared to AA and AB genotypes. *LHβ* gene was found to be polymorphic (**Reen et al., 2018**) and total six SNPs were identified in *LHβ* gene (C356090A, C356113T, A356701G, G355869A, G356330C, and G356606T). Single Stranded Conformational Polymorphism (SSCP) variants of pattern 2 of exon 1 + pattern 2 of exon 2 + pattern 1 of exon 3 had highly significant effect on sperms concentration (million/*ml*), percent mass motility, acrosome integrity, membrane integrity ($p < 0.01$) and percent live spermatozoa ($p < 0.05$).

Nikbin et al. (2018) detected the associations of four SNPs in caprine *FSHβ* and *LHβ* with quality traits of fresh and post-thaw semen of male goats and revealed that there were significant associations of the candidate genes with libido and semen quality traits and the three SNPs of *FSHβ*3 had significant effects on libido, progressive motility, and abnormality of fresh semen ($P < 0.05$), motility, velocity, and viability traits of post-thaw semen ($P < 0.05$) and *LHβ*2 polymorphism only showed association with sperm viability of post-thaw semen.

3. MATERIALS AND METHODS

3.1 A crossbreeding experiment performed and animals used:

A crossbreeding experiment between APRI line bucks (A) with Moshtohor line does (M) was carried out in the rabbitry of the Faculty of Agriculture, Benha University, Egypt to get $\frac{1}{2}A\frac{1}{2}M$ cross. This experiment was conducted during the period from September 2015 until December 2017 to study the crossbreeding effects on semen traits. The animals used in this experiment were Moshtohor line, APRI line, $\frac{1}{2}A\frac{1}{2}M$ and V line as a reference population. APRI line was developed in the Animal Production Research Institute, Ministry of Agriculture, Egypt (Youssef et al., 2009). The synthesis was conducted by crossing Red Baladi bucks (B) with V line does (V) followed by selection for litter weight at weaning in three subsequent generations and after F3, the animals were called APRI line consisting of $\frac{1}{2}B\frac{1}{2}V$. Moshtohor line was established in the Department of Animal Production, Faculty of Agriculture, Moshtohor, Benha University, Egypt (Iraqi et al., 2008). The synthesizing pattern was similar to that for APRI line, but with using Sinai Gabali bucks (S) instead of Red Baladi bucks and forming $\frac{1}{2}S\frac{1}{2}V$ where selection was practiced for litter weight at weaning and individual weight at 56 d and the animals were kept in the rabbitry of Benha University. The V line is a maternal line selected for litter size at weaning by Animal Science Department, Universidad Politécnica de Valencia, (UPV), Valencia, Spain. The interest of using line V lies in several features; first it has long history of selection in Valencia where the climate is not widely different from the weather of Delta of

Nile in Egypt (**García and Baselga, 2002**), second it has good performance in Saudi Arabia and had confirmed the fact that it has adaptable pattern in heat stress conditions (**Khalil et al., 2005**). There are two Sinai Gabali breeds of rabbits in Egypt bearing the name ‘Sinai Gabali, the first one was originated in the western desert of the north Mediterranean coast, while the second one was originated in Sinai Peninsula, the two strains seem to be acclimatized to the desert conditions.

3.2 Mating practiced:

Mating was occurred at random within each genetic group, avoiding full and half sibs matings as well as parent offspring matings. The total number of weaned bunnies, sires and dams were 1201, 179 and 261, respectively (**Table 12**). Age of the does and bucks ranged between 4.5 and 5 months at first time of mating. Culled bucks and does or dead ones during the experimental period were replaced randomly by their replacements from the same line and from the original stock. The ratio of the experimental buck to does was 1:3 by using natural mating. Sire-daughter, full and half-sibs matings were avoided. Each doe was transferred to the assigned buck to be mated and returned back again to her own cage. On the day 10 post mating, each doe was palpated to detect pregnancy. Doe that was not pregnant would returned to the same mating buck to be re-mated and returned every other day thereafter until a service was observed. Likewise, does were re-mated after kindling by the same assigned buck.

Table 12: Number of animals, sires and dams categorized according to their genetic groups

Animal genetic group	Buck genetic group	Doe genetic group	Number of bunny weaned	Number of sires used	Number of dams used
V	V	V	439	60	90
A	A	A	272	47	58
M	M	M	312	48	73
½A½M	A	M	178	24	40
Total			1201	179	261

V=V line, A=APRI line, M=Moshtohor line.

3.3 Housing and feeding:

The rabbits were raised in one floor rabbitry, oriented east to west where windows and fans were oriented to control the ventilation. The rabbitry was equipped with regular electric heaters in winter to keep the minimum degree of temperature at 15°C. In summer, there are fans and forced air-cooling to keep the maximum temperature at 35°C. Breeding bucks and does were housed individually in wired cages with standard dimensions (60 x 40 x 35 cm of length x width x height) and arranged in flat deck batteries. In the rabbitry, temperature ranged from 15 to 19°C in winter with the relative humidity ranged from 40 to 80 % and photoperiod was at 16 L: 8 D. Each maternity cage was supplied with a galvanized steel nest boxes. Cages and nest boxes were cleaned and disinfected regularly after each kindling and urine and manure were cleaned every day morning. On the 25th day of pregnancy, the nest boxes were supplied with thick layer of rice straw, which was placed in the bottom of the nest box to help the doe in preparing a warm comfortable nest for her bunnies. Litters were examined and counted

within 12 hours after kindling and were checked and examined every morning during the suckling period to remove the dead bunnies. The bunnies were weaned, sexed, ear tagged, and transferred to standard progeny wire cages at 28 days post kindling.

The animals were fed *ad libitum* all over the experimental period on a pelleted commercial ration; pellets were cylindrical in form (1-2 cm in length and 0.4 cm in diameter). The ration was composed of 23 % barley , 19 % wheat bran , 24 % soybean meal , 21 % berseem hay , 13 % yellow corn , 1 % limestone, 0.5 % table salt, 14 kg di-calcium phosphate/ton, 1 kg minerals mixture/ton, 1 kg anti-coccidian/ton, 1 kg anti-toxicity/ton, provided 18.01% crude protein, 13.7% crude fiber and 2.5% fat (digestible energy = 2500 to 2700 kc/kg feed). Berseem "*trifolium alexandrinum*" was provided in winter months to all animals to increase milk production of does and to improve livability. All the animals were kept in the same house and reared under the same environmental conditions in every stage of productivity. Fresh drinking water was available all the time.

3.4 Data of growth traits:

Data were recorded for individual rabbits body weights (BW) in four genetic groups (V line, APRI line, M line and ½A½M cross) at 4, 6, 8, 10, and 12 weeks of age. Daily weight gains (DG) were calculated during the age intervals from 4-6, 6-8, 8-10 and 10-12 weeks of age.

3.5 Semen collection and evaluation:

A total number of 1050 ejaculates collected from 149 bucks were evaluated for semen characteristics (**Table 13**). Semen was collected during morning using Artificial Vagina (AV) according to **Bredderman et al. (1964)** and **Khalil et al. (2007)**. The experiment was designed to study some semen quality traits in five genetic groups (V line, APRI line, M line, Sinai Gabali and ½A½M cross). Bucks were housed in individual cages and all the bucks were trained for semen collection by using Artificial Vagina where the female rabbit was applied as a teaser. After three weeks of adapting the bucks for semen collection, semen was collected from each buck separately once morning per week.

Table 13: Number of bucks and ejaculates categorized according to the genetic group.

Buck genetic group	No. of bucks used	No. of ejaculates obtained
V Line	36	275
M Line	28	239
A Line	42	312
½A½M	23	144
Sinai Gabali	20	80
Total	149	1050

A=APRI-line, M=Moshtohor-line.

The artificial vagina firstly was filled with 40°C warm water through the water opening. Secondly, the artificial vagina is filled with air through the air opening. The artificial vagina was lubricated with vaslin using glass rod then it placed between the hind legs of the does. The female with artificial vagina were transferred to the male cage, then semen could be easily collected. Semen was kept in

water bath at 37°C immediately after collection until evaluation. Each ejaculate was evaluated manually and under the microscope. The ejaculate semen volume was measured per *ml* using a graduated tube directly after collection. The *pH* of semen samples was specified just after collection using *pH* indicator paper ranging from 6.0 to 8.1 with 0.3 grades (Whatman *pH* Indicator paper; Whatman Limited Maidstone, England). Percentage of progressive motility of spermatozoa was estimated immediately after each collection using microscopic screening and by placing a small drops of fresh semen on a warm glass slide (37°- 38°), then diluted with two drops of warm 0.9% NaCl and enveloped with a cover slip. Semen examination was made under high power microscope (x400) and inspection was made under high power enlargement (x675). Discrimination between live and dead spermatozoa was estimated by Eosin - Nigrosin stain technique. Reiterates semen were made and a total of 200 spermatozoa were enumerated per each slid using the oil Seder (x1350). The live spermatozoa were spotless, while the dead spermatozoa were soiled. One smear from each ejaculate was soiled with Eosin-Nigrosin stain and a total of 100 sperms were examined randomly. The percentage of normal sperms was predestined according to **Baril et al. (1993)**, normal sperm as % = 100 – abnormal sperm as %. Sperm cell concentration ($\times 10^6/ml$) was quantified by direct cell count using the improved Neubauer haemocytometer.

Materials And Methods

3.6 Statistical models for estimating variance components and crossbreeding genetic effects:

Data of body weights and daily weight gains were analyzed using the following multi-trait animal model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_a\mathbf{u}_a + \mathbf{e} \quad (\text{Model 1})$$

Where \mathbf{y} = vector of the observed growth trait for the weaned rabbit; \mathbf{b} = vector of the fixed effects of genetic group of progeny (four levels, see Table 12), sex (males and females), year-season of birth (eight levels), parity (five levels) and litter size in which the animal was born (three levels); \mathbf{X} and \mathbf{Z}_a are incidence matrices corresponding to fixed and random additive effects of the rabbit (\mathbf{u}_a), respectively; \mathbf{e} = vector of random residual effects.

Data of semen quality traits were analyzed using the following multi-trait animal model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_a\mathbf{u}_a + \mathbf{Z}_p\mathbf{u}_p + \mathbf{e} \quad (\text{Model 2})$$

Where \mathbf{y} = vector of observed semen parameter for the buck; \mathbf{b} = vector of fixed effects of genetic group of the buck (five levels, see Table 13), year-season of semen collection (8 year-season levels) number of ejaculates (six ejaculates of buck); \mathbf{u}_a = vector of random additive effect of the bucks and sires and dams of bucks; \mathbf{u}_p = vector of random effects of the permanent non-additive effect of the bucks; \mathbf{X} , \mathbf{Z}_a and \mathbf{Z}_p = incidence matrices relating records to the fixed effects, additive genetic effects and permanent environment, respectively; \mathbf{e} = vector of random residual effects.

The variance components of random effects and heritabilities were estimated by two softwares of VCE6 (Groeneveld et al., 2010) and TM that used a Bayesian inference of Gibbs sampling algorithm

(Legarra et al., 2008). The variance components were used to solve the corresponding mixed model equations, obtaining solutions for the genetic group effects and their error variance–covariance matrix using the PEST software (Groeneveld, 2006). According to the theory of Dickerson (1992), the solutions of the crossbreeding genetic group effects were obtained using the procedure of Generalized Least Squares (GLS) and applying the following linear model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{e}, \text{Var}(\mathbf{y}) = \mathbf{V} \quad (\text{Model 3})$$

Where: \mathbf{y} = vector of the estimated genetic groups solutions; \mathbf{X} = incidence matrix; \mathbf{b} = vector of estimable crossbreeding genetic effects; \mathbf{e} = vector of random error; \mathbf{V} = the error variance-covariance matrix of \mathbf{y} .

The coefficients relating the genetic crossbreeding effects to the means of the genetic groups (Table 14) were estimated according to Dickerson (1992) and using CBE software of Wolf (1996). The crossbreeding parameters representing the differences between the genetic groups were estimated in terms of direct additive effects ($G^I = G_A^I - G_M^I$), maternal additive effects (G^M) and direct heterosis (H^I). Thus, we have three parameters to be estimated (the vector called \mathbf{b} -vector):

$$\mathbf{b} = [(G_A^I - G_M^I) \ G^M \ H^I]$$

The solutions of \mathbf{b} were calculated by the method of Generalized Least Squares (GLS) using the following equation:

$$\hat{\mathbf{b}} = (\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{X}'\mathbf{V}^{-1}\mathbf{y}$$

Where \mathbf{X} was the matrix of coefficients of estimable crossbreeding effects, \mathbf{V}^- = the generalized error variance–covariance matrix, with the variance–covariance matrix of the estimate of \mathbf{b} being,

$$\text{Var}\hat{\mathbf{b}} = (\mathbf{X}' \mathbf{V}^- \mathbf{X})^{-1}$$

The matrix used to test the significance of the crossbreeding effects is presented in **Table 14**.

Table 14: Genetic groups of animals with their sires and dams and coefficients of the matrix relating the means of the genetic groups with crossbreeding effects

Genetic group			Mean	Coefficients of the matrix				
Animal	Sire	Dam		G_A^P	G_M^P	G_A^M	G_M^M	H^I
A	A	A	1	1	0	1	0	0
M	M	M	1	0	1	0	1	0
$\frac{1}{2}A\frac{1}{2}M$	A	M	1	0.5	0.5	0.5	0.5	1

G_A^P and G_M^P = Direct additive genetic effects for APRI and Moshtohor lines, respectively; G_A^M and G_M^M = Maternal genetic effects for the APRI and Moshtohor lines, respectively; H^I = Direct heterosis.

3.7 Molecular genetic analyses:

3.7.1 Blood sampling and DNA extraction:

For the molecular analyses, 149 bucks belong to V line, APRI line, Moshtohor line, Sinai Gabali and $\frac{1}{2}A\frac{1}{2}M$ cross were used in this study. The samples were collected from animals with the least relationship with avoiding full sibs to decrease the genetic relationship between the genotyped animals. Approximately 3-5 ml venous blood sample per animal was obtained from the rabbit ear vein by 2-gauge 1.5-injection needle into tubes containing EDTA as anticoagulant. The blood samples were preserved in ice tank till reaching the laboratory and were then kept in freezer at -20 °C. Genomic DNA was extracted from leukocytes using the Gene Jet

Whole Blood Genomic DNA purity Mini Kit (Cat No. #K0781, Thermo Scientific). A 20 μ l of proteinase K solution was added to 200 μ l of whole blood in 2 *ml* eppendorf tube, and mixed by vortexing; then 300 μ l of lysis solution was added and mixed thoroughly by pipetting to obtain a uniform suspension. The sample was incubated at 56°C for 10 minutes using a shaking water bath until the cells were completely lysed. A 200 μ l of ethanol (96-100%) was added and mixed by pipetting. The prepared mixture was transferred to the spin column. Then, centrifuged at 6000 rpm for 1 minute at room temperature and remove the collection tube was containing the flow-through solution. The spin column was placed into a new 2 *ml* collection tube; then 500 μ l of wash buffer I were added and centrifuged at 8000 rpm for 1 minute at room temperature. The flow-through was discarded and the column placed back into the collection tube. A 500 μ l of wash buffer II was added to the column and centrifuged at 16000 rpm for 3 minute at room temperature. The collection tube was emptied and the purification column placed back into the tube and re-spined the column at 16000 rpm for 1 min. The collection tube containing the flow-through solution was discarded and transferred the column to a sterile 1.5 *ml* microcentrifuge tube. A 200 μ l of elution buffer was added to the center of the column membrane to eluted genomic DNA and incubated for 2 min and centrifuged at 8000 rpm for 1 minute at room temperature. Genomic DNA was stored at – 20°C. Then, high quality and purified and concentrated DNA products were obtained to be used directly in a variety of downstream applications.

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3.7.2 Amplification by polymerase chain reaction (PCR):

On chromosome 19, NC_013687.1, PCR was done for amplification of part of the 5'untranslated region and part of exon 1 of the growth hormone gene (*GH*) with expected amplicon size of 231 bp and annealing Temperature per time of 58/30 (°C per S). According to **Fontanesi et al. (2012)**, the primer sequence and PCR-RFLP assay used in the amplification were: the forward primer 5'-GTATAGTGGGATGGGGTTGG -3' and the reverse primer 5'-TTACGCTCCCATTTCAGAAGC -3. PCR amplifications were carried out in 50 µl reaction mixture composed of 4 µl genomic DNA (100 ng/µl) as a template, 10 pmol of each primer, 2mM dNTP' (dATP, dCTP, dTTP and dGTP; ABgene, Surrey, UK), 10X PCR buffer, 25 mM MgCl₂, and 1 unit Taq DNA polymerase. A master mix was prepared in a 2 ml tube according to the number of PCR reactions to be performed, with an extra reaction included to compensate the loss part of the solution due to frequent pipetting. An aliquot of 46 µl master mix solution was dispensed in each PCR tube (0.2 ml tube), containing 4 µl of the appropriate template DNA. Accordingly, the PCR reaction components are presented. Thermal cycling was carried out by initial denaturation at 95°C for 5 min, followed by 35 cycles each at 95°C for 30 seconds, annealing temperature at 58°C for 30 seconds, extremism temperature at 72°C for 30 seconds and final extension at 72°C for 10 min., then the samples were held at 4°C. The amplified DNA fragments were separated on 2 % agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by gel

documentation system (Alpha Imager M1220, Documentation and Analysis, System, Canada).

3.7.3 Genotyping using PCR-RFLP technique:

The c.-78C>T SNP of the growth hormone gene (**Fontanesi et al., 2012**) was genotyped by PCR-RFLP using *Bsh1236I* (*BstUI*) restriction enzyme (Fermentas, Vilnius, Lithuania). The RFLP technique was carried out in reaction volume 20 µl consisted of: 7 µl H₂O, 2 µl buffer, 10 µl PCR product and 1 µl restriction enzyme. Digested fragments were visualized by electrophoresis on 3 % agarose gel stained with ethidium bromide at 100 V in 1x TAE and visualized on a UV Transilluminator and photographed by gel documentation system. The 50 bp DNA step ladder (Promega) was included in each run.

3.7.4 Characterizing the molecular data in different genetic groups:

Allelic and genotypic frequencies were calculated and the genetic diversity of SNP C>T, located in the exon 1 region of *GH* gene was assessed by calculating the effective number of alleles (*Ne*) and the observed (*Ho*) and the expected (*He*) heterozygosity using GENALEX program, version 6.5 (**Peakall and Smouse, 2006**):

$$Ne = \frac{1}{\sum_{i=1}^n p_i^2} \quad Ho = \frac{\text{No. of heterozygosity}}{n} \quad He = 1 - \sum_{i=1}^n p_i^2$$

Where P_i = the frequency of the i th allele, P_j = the frequency of the j th allele and n = the number of alleles. Hardy-Weinberg equilibrium

(HWE) within each population was estimated using GENEPOP program (Raymond, 1995); <http://genepop.curtin.edu.au/>) performing the Chi-Square test for each genetic group studied. The polymorphism information content (PIC, as specified below) was calculated using CERVUS software, version 3 (Kalinowski et al., 2007):

$$PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} - \sum_{j=i+1}^n 2p_i^2 2p_j^2$$

Where PIC, P_i and P_j as defined previously

3.7.5 Model for detecting the associations between genotypes of GH gene and growth traits:

For the associations between the SNP genotypes of *GH* gene and post-weaning growth traits in each genetic group separately, the effects of genotype C/T SNP on different growth traits were estimated using PEST software (Groeneveld, 2006) and applying the same multi-traits animal model defined in **Model 1** (in matrix notation) after adding the fixed effect of the i^{th} C/T SNP genotype of *GH* gene (three genotypes; TT, CC and TC). The method of Generalized Least Squares (GLS) was used to get the polymorphic associations between genotypes of *GH* gene and growth traits.

3.7.6 Model for detecting the associations between genotypes of GH gene and semen traits:

For the associations between the genotypes of *GH* gene and semen traits in each genetic group separately, the effects of genotype C/T SNP on different semen traits were estimated using PEST

software (**Groeneveld, 2006**) and applying the same multi-trait animal model defined in **Model 2** (in matrix notation) after adding the fixed effect of the i^{th} C/T SNP genotype of *GH* gene (three genotypes of TT, CC and TC). The method of Generalized Least Squares (GLS) was used to get the polymorphic associations between genotypes of *GH* gene and semen traits.

4. RESULTS AND DISCUSSION

4.1 Growth traits (body weights and daily gains):

4.1.1 Actual means and variations:

Actual means, standard deviations (SD) and coefficients of variation (CV%) for body weights at 4, 6, 8, 10 and 12 weeks of age and daily weight gains (DG) during the intervals from 4 to 6, 6 to 8, 8 to 10 and 10 to 12 weeks of age across all genetic groups were presented in **Table 15**. The overall means of body weights were 490, 780, 1085, 1330 and 1670 g at 4, 6, 8, 10 and 12 weeks of age and daily weight gains were 20.5, 21.8, 18.2 and 24.2 g at 4-6, 6-8, 8-10 and 10-12 weeks, respectively. **Youssef et al. (2009)** estimated that the average body weights of APRI line at 4, 6, 8, 10 and 12 weeks were 501, 740, 1028, 1323 and 1651g and the daily weight gains were 17.0, 20.5, 20.9 and 23.2 g at intervals of 4-6, 6-8, 8-10 and 10-12 week, respectively.

Table 15: Actual means, standard deviations (SD), coefficients of variation (CV), and ranges for growth traits across all genetic groups

Trait	Number of records	Mean	SD	CV	Minimum	Maximum
Body weights at:						
4 weeks (g)	1201	490	60.93	12	310	685
6 weeks (g)	1164	780	80.43	10	530	1010
8 weeks (g)	1116	1085	112.16	10	760	1350
10 weeks (g)	1058	1330	96.22	7	1000	1820
12 weeks (g)	984	1670	138.46	8	1230	2000
Daily weight gains at:						
4-6 weeks (g)	1163	20.51	7.04	34	1.4	50
6-8 weeks (g)	1115	21.84	8.56	39	0.7	45.7
8-10 weeks (g)	1046	18.17	7.91	44	0.7	52.1
10-12weeks (g)	979	24.16	9.20	38	0.7	57.1

The ranges between minimum and maximum values in body weights at 4, 6, 8, 10 and 12 weeks across all genetic groups of rabbits were high, being 310 to 685, 530 to 1010, 760 to 1350, 1000 to 1820 and 1230 to 2000 g, respectively and from 0.7 to 57.1 g for daily gains. The coefficients of variation for body weights were moderate, ranging from 7 to 12%, while the coefficients for daily weight gains were high ranging from 34 to 44%.

4.1.2 Body weights and daily weight gains in different genetic groups:

Generalized least square means of body weights in $\frac{1}{2}A\frac{1}{2}M$ cross were higher than APRI line and lower than Moshtohor line at 4, 6, 8, 10 and 12 weeks of age (**Table 16**).

Table 16: Generalized least square means \pm standard errors (SE) for body weights (BW) and daily weight gains (DG) as affected by genetic groups

Traits	Genetic groups							
	V line		M line		A line		$\frac{1}{2}A\frac{1}{2}M$	
	GLM	SE	GLM	SE	GLM	SE	GLM	SE
Body weights at:								
BW4	475 ^d	15.8	520 ^a	20.1	490 ^c	18.8	505 ^b	21.38
BW6	780 ^b	29.6	800 ^a	27.3	755 ^d	25.7	775 ^c	29.17
BW8	1125 ^a	40.6	1085 ^b	37.2	1040 ^c	35.0	1050 ^c	40.15
BW10	1325 ^b	89.6	1370 ^a	78.8	1310 ^d	74.0	1320 ^c	85.8
BW12	1665 ^b	52.6	1690 ^a	49.2	1655 ^d	46.4	1660 ^c	52.4
Daily weight gains at:								
DG4-6	22.1 ^a	0.91	20.2 ^b	1.36	18.9 ^d	0.94	19.5 ^c	1.05
DG6-8	24.1 ^a	1.17	20.8 ^b	1.18	20.5 ^b	1.21	20 ^c	1.35
DG8-10	15.4 ^d	1.12	20.2 ^a	1.13	19.4 ^b	1.16	18.9 ^c	1.29
DG10-12	23.9 ^b	1.35	23.6 ^c	0.92	23.9 ^b	1.4	24.9 ^a	1.56

A=APRI line, M=Moshtohor line, Means within row, not sharing any letter, are significantly different ($P<0.05$).

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These averages were lower than those values obtained for V line reared in Spain (e.g. **García et al., 2000**). These averages of ½A½M cross rabbits were higher than those of APRI line intensified in Egypt (**Youssef et al., 2009**). Moreover, body weight at 4, 8 and 12 week of ½A½M cross rabbits were in agreement with **Iraqi et al. (2008)** who found that the averages body weights at 4, 8 and 12 weeks of age were 589, 1193 and 1676g respectively, in Moshtohor line rabbits.

For daily weight gains, the averages were mostly moderate and ranged from 15.4 to 24.9 g (**Table 16**). These results were in agreement with **Iraqi et al. (2008)** who recorded an average daily gain of Moshtohor line in Egypt to be 17.5 to 21.2 g/day. In Spain, V line rabbits were higher, being 36.5 g/day for daily gain between weaning and 8 weeks and 37.9 g/day between weaning and 9 weeks of age (**García et al., 2000**). **Youssef et al. (2009)** reported that APRI line rabbits in Egypt were lower in average daily gain than those of crossed rabbits obtained here (17.0 to 23.2 g/day).

4.1.3 Heritabilities (h^2):

Heritabilities for body weights and gains estimated by threshold model (TM) and VCE6 softwares are presented in **Table 17**. Heritabilities estimated by VCE program for body weights were mostly low or moderate and ranging from 0.06 to 0.18, while the estimates for daily body gains ranged from 0.05 to 0.10. The heritabilities estimated by TM program for body weights were mostly low or moderate and ranging from 0.09 to 0.18, while the estimates for daily weight gains ranging from 0.07 to 0.11. Accordingly,

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heritabilities estimated by VCE and MT programs are nearly similar. However, the accuracy of estimation was higher for TM compared to VCE6, since, the standard errors of estimates resulted from TM were lower. The heritabilities obtained here for body weights and gains were nearly similar to those estimates obtained by some studies in Egypt (Youssef, 2004; Youssef et al., 2009), in Nigeria (Akanno and Ibe, 2005), and in Brazil (Ferraz and Eler, 1996). Published estimates of heritability for daily weight gains were higher than those obtained in this experiment (Piles et al., 2004b). Khalil and Al-Homidan (2014) reported that the heritabilities were mostly moderate and ranged from 0.19 to 0.27 for body weights and from 0.18 to 0.34 for daily gains. Iraqi (2008) estimated the heritability in Sinai Gabali rabbits to be 0.05, 0.38 and 0.20 for body weights at 4, 8 and 12 weeks, respectively and 0.23 and 0.19 for daily weight gains at intervals of 4-8 and 8-12 weeks, respectively.

Table 17: Estimates of heritability \pm standard errors (SE) for growth traits estimated by TM and VCE6 softwares

Trait	Number of records	($h^2 \pm$ SE estimated by VCE6)	($h^2 \pm$ SE estimated by TM)
Body weights at:			
4 weeks	1201	0.10 \pm 0.033	0.11 \pm 0.05
6 weeks	1164	0.18 \pm 0.035	0.15 \pm 0.05
8 weeks	1116	0.17 \pm 0.039	0.18 \pm 0.06
10 weeks	1058	0.06 \pm 0.037	0.10 \pm 0.05
12 weeks	984	0.08 \pm 0.043	0.09 \pm 0.04
Daily weight gains at:			
4-6 weeks	1163	0.08 \pm 0.001	0.10 \pm 0.04
6-8 weeks	1115	0.10 \pm 0.002	0.11 \pm 0.04
8-10 weeks	1046	0.05 \pm 0.003	0.07 \pm 0.05
10-12 weeks	979	0.07 \pm 0.002	0.10 \pm 0.05

TM= Threshold Model; VCE6= Variance Component Estimation.

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4.1.4 Crossbreeding analysis:

4.1.4.1 Direct additive effects (G^I):

The generalized least square solutions of G^I and their percentages for body weights and gains given in **Table 18** showed that the estimable solutions of G^I were significantly in favour of Moshtohor line rabbits by 13.5 to 48.1g for body weights and 0.5 to 3.7g for daily gains, with percentages ranging from 1.4 to 4.3% for body weights and 2.1 to 18.6% for daily weight gains ($P < 0.01$). However, crossbreeding experiments carried out in Egypt indicated that direct additive effects on most post-weaning body weights and gains were significant (**Khalil and Khalil, 1991; Youssef et al., 2009; Ibrahim et al., 2008**). **Khalil and Khalil (1991)** reported significant estimates of G^I for body weight at 12 weeks of age. **Abdel-Ghany et al. (2000b)** noted that direct additive effects from crossing New Zealand White with Baladi Red or Baladi Black rabbits were consistently in favour of Baladi Red or Baladi Black for post-weaning body weights and gains. In crossing V line and Baladi Red rabbits, **Youssef et al. (2009)** found that the direct additive effects were in favour of V line rabbits reaching 15.0% at 4 weeks and 13.3% at 12 weeks and the effects for daily body gains were significant reaching 35.7% at the interval of 10–12 weeks. In crossing Saudi Gabali with V line rabbits, the direct additive effects were significantly in favour of V line and ranging from 2.7 to 8.1% for body weights and 3.8 to 13.1% for daily weight gains (**Khalil and Al-Homidan, 2014**).

Likewise, **Piles et al. (2004b)** in crossing C line and R line rabbits in Spain reported significant estimates of direct additive

effects for body weights and gaily gains at 32-60 days. **Ouyed and Brun (2008b)** in crossing Californian with New Zealand White found that the estimates of G^I were in favour of New Zealand White for 63-d body weight.

Table 18: Generalized least square solutions and percentages of direct additive effects ($G^I = G_A^I - G_M^I$) and their standard errors (SE) for body weights and gains in crossing APRI and Moshtohor lines

G^I for body weights (BW)					G^I for daily weight gains (DG)				
Trait	N	G^I solution (units)	SE	G^I as % ⁺	Trait	N	G^I solution (units)	SE	G^I as % ⁺
BW4	762	-13.5**	0.11	-2.7					
BW6	729	-33.2**	0.13	-4.3	DG4-6	762	-1.7**	0.025	-8.7
BW8	699	-14.8**	0.16	-1.4	DG6-8	697	-3.3**	0.029	-15.9
BW10	672	-35.2**	1.4	-2.6	DG8-10	611	-3.7**	0.032	-18.6
BW12	628	-48.1**	1.2	-2.9	DG10-12	611	-0.5 ^{NS}	0.033	-2.1

⁺Percentage computed as [Estimate of G^I in units / (A+M)/2] x100

NS= non-significant, **= $P \leq 0.01$

4.1.4.2 Maternal effect (G^M):

The solutions of maternal effects and their percentages for body weights and gains given in **Table 19** showed that the estimable solutions of G^M were significantly in favour of Moshtohor line by 14.2 to 51.8 g for body weights and 0.48 to 3.1g for daily weight gains in weight, with percentages ranging from 2.8 to 5.4% for body weights and 1.8 to 15.0% for daily gains. Similarly, crossbreeding experiments carried out in Egypt indicated that maternal effects on most body weights and gains were significant (e.g. **Abou Khadiga et al., 2008; Ibrahim et al., 2008; Iraqi et al., 2008; Hekil et al., 2011**). **Abdel-Ghany et al. (2000a)** reported that maternal additive

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effects on body weights and daily weight gains were significantly in preference of New Zealand White at all ages considered, While **Abou Khadiga et al. (2008)** found that maternal genetic effects were significantly in favor of V line dams for body weights at 8 and 12 weeks of age. **Iraqi et al. (2008)** found that Gabali breed was significantly superior in G^M over V line ($P<0.01$) for body weights at 8 and 12 weeks of age and daily weight gains at intervals of 4-8 and 8-12 weeks of age.

Table 19: Generalized least square solutions and percentages of maternal effects ($G^M = G^M_A - G^M_M$) and their standard errors (SE) for body weights and gains in crossing APRI and Moshtohor lines

G^M for body weights (BW)					G^M for daily weight gains (DG)				
Trait	N	G^M solution (units)	SE	G^M as % ⁺	Trait	N	G^M solution (units)	SE	G^M as % ⁺
BW4	762	-14.2**	0.09	-2.8					
BW6	729	-42.4**	0.11	-5.4	DG4-6	762	-1.02*	0.018	-5.2
BW8	699	-37.2**	0.13	-3.5	DG6-8	697	-3.1**	0.024	-15.0
BW10	672	-47.3**	0.8	-3.5	DG8-10	611	-2.9**	0.026	-14.6
BW12	628	-51.8**	1.01	-3.1	DG10-12	611	-0.48 ^{NS}	0.024	-1.8

⁺Percentage computed as [Estimate of G^M in units/ (A+M)/2] x100

NS= non-significant, *= $P\leq 0.05$ and **= $P\leq 0.01$

4.1.4.3 Direct heterosis (H^I):

The generalized least square solutions of H^I and their percentages for body weights and gains given in **Table 20** indicated that the estimable solutions of direct heterosis were positive and highly significant and ranged from 15.5 to 87.1g for body weights and from 0.2 to 2.7g for body gains, with percentages ranging from 3.1 to 8.2% for body weights and 1.1 to 13.0% for daily gains. **Afifi et al. (1994)** found that heterosis percentages in crossing New

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Zealand White with Baladi rabbits in Egypt were positive and ranged from 2.7 to 9.5% for post-weaning body weights and daily gains. **Iraqi et al. (2008)** found that the estimates of heterosis were always significantly positive by 6.9, 3.6 and 5.4% for body weights at 4, 8 and 12 weeks respectively and 9.7 and 6.1% for daily weight gains at intervals of 4-8 and 8-12 weeks, respectively. **Youssef et al. (2009)** found that direct heterosis percentages were positive and ranged from 4.9 to 16.7% for body weights and 14.4 to 29.5% for daily gains. **Khalil and Al-Homidan (2014)** reported that direct heterosis were significantly positive and ranged from 4.5 to 5.4 % for body weights and from 6.6 to 9.6 % for daily gains.

Table 20: Generalized least square solutions and percentages of heterotic effects ($H^I = H_A^I - H_M^I$) and their standard errors (SE) for body weights and gains in crossing APRI and Moshtohor lines

H ^I for body weights (BW)					H ^I for daily weight gains (DG)				
Trait	N	H ^I solution (units)	SE	H ^I as % ⁺	Trait	N	H ^I solution (units)	SE	H ^I as % ⁺
BW4	762	15.5**	0.16	3.1					
BW6	729	62.6**	0.19	8.1	DG4-6	762	0.2 ^{NS}	0.026	1.1
BW8	699	87.1**	0.24	8.2	DG6-8	697	2.7**	0.044	13.0
BW10	672	70.5**	1.3	5.2	DG8-10	611	1.6*	0.005	7.8
BW12	628	63.9**	2.1	3.8	DG10-12	611	2.3**	0.049	9.6

⁺Percentage computed as [Estimate of H^I in units/ (A+M)/2] x100

⁺⁺NS= non-significant, *=P≤0.05 and **=P≤0.01

4.2 Semen traits:

4.2.1 Actual means and variations:

Actual means, standard deviations (SD) and coefficients of variation (CV%) for semen quality traits of ejaculate volume, pH of semen, motility of sperms, live sperms, dead sperms, normal sperms,

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abnormal sperms and concentration of sperms of bucks across the five studied genetic groups were presented in **Table 21**. The overall means of semen traits were 0.65 *ml*, 7.53, 49.91 %, 80.5 %, 19.4 %, 86.8 %, 13.18 % and 445.9 x 10⁶/*ml* for VE, *pH*, MS, LS, DS, NS, AS and SC, respectively. Values of most semen traits obtained in the present study were similar to those obtained in literature (**Alvariño, 2000; Arroita et al., 2000; Al-sobayil and Khalil, 2002; García-Tomás et al., 2006; Iraqi et al., 2012**). In Saudi Arabia, **Khalil et al. (2007)** estimated that the average semen traits of VE, *pH*, MS, DS, AS and SC of the cross between V line and Gabali Saudi rabbits to be 0.63, 7.5, 65.7 %, 8.1 %, 14.8 % and 434 x10⁶/*ml*, respectively.

Table 21: Actual means, standard deviations (SD), coefficients of variation (CV) and ranges for semen traits across all genetic groups

Semen trait	Mean	SD	CV%	Minimum	Maximum
Volume of ejaculate (<i>ml</i>)	0.65	0.24	36	0.1	1.4
<i>pH</i> of semen	7.53	0.51	7	6.2	8.7
Motility of sperms %	49.91	15.1	30	10	90
Live sperms %	80.58	8.19	10	18	96
Normal sperms %	86.81	5.7	7	10	98
Concentration of sperms X10 ⁶ / <i>ml</i>	445.9	191	43	60	965
Dead sperms %	19.41	8.2	42	4	82
Abnormal sperms %	13.18	5.7	43	2	90

Number of records= 1050

The ranges between minimum and maximum values in semen traits of across all studied genetic groups of rabbits were high, being 0.1 to 1.4, 6.2 to 8.7, 10 to 90 %, 18 to 96 %, 10 to 98 % and 60 to 965 x10⁶/*ml*, 4 to 82% and 2 to 90% in VE, *pH*, MS, LS, NS, SC, DS and AS, respectively. **Khalil et al. (2007)** in Saudi Arabia stated that the minimum and maximum values in semen traits were high being

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0.1 to 1.5 *ml*, 5.5 to 9.0, 5.0 to 95.0 %, 0.0 to 40.0 %, 0.0 to 45.0 % and 5.0 to 1080 $\times 10^6/ml$ for VE, *pH*, MS, DS, AS and SC, respectively in V line and Gabali Saudi rabbits and their crosses.

4.2.2 Semen traits in different genetic groups:

Generalized least square means of semen traits in $\frac{1}{2}A\frac{1}{2}M$ cross were higher than APRI line and lower than Moshtohor line in terms of volume of ejaculate, *pH* of semen, motility of sperms, live sperm, normal sperms, abnormal sperms and concentration of sperms (**Table 22**).

The ejaculate volume of V line (0.64 *ml*) was higher than that of Sinai Gabali (0.54 *ml*). The averages of ejaculate volume were mostly moderate and ranged from 0.54 to 0.69 *ml* and they were nearly similar to that observed by **Iraqi et al. (2012)** who reported significant differences between Sinai Gabali, V line and Moshtohor line rabbits, with values being 0.60, 0.66 and 0.72 *ml*, respectively.

The semen *pH* of V line (7.9) was higher than that of Moshtohor line (7.6), Sinai Gabali (7.6), $\frac{1}{2}A\frac{1}{2}M$ cross (7.5) and APRI line (7.4) rabbits (**Table 22**) These results were in agreement with **Al-Sobayil and Khalil (2002)** who reported that differences between V line and Sinai Gabali rabbits were significant (7.12 and 5.47) for semen *pH*. **El-Azim and El-Kamash (2011)** showed significant differences in *pH* of semen between Californian (7.5), New Zealand White (7.42), Sinai (7.33) and Baladi (7.35) rabbits.

The sperm motility in semen was higher in Moshtohor line (50.7%) and $\frac{1}{2}A\frac{1}{2}M$ cross (51.6%) than Sinai Gabali, APRI line and V line which recorded values of 47.3, 47.2, 49.2%, respectively

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(**Table 22**). These figures were higher than sperms motility of Sinai Gabali (46.9%), V line (47.2%), and Moshtohor line (50.7%) recorded by **Iraqi et al. (2012)**.

The percentages of live sperms in V line (82.4%) and Sinai Gabali (82.1%) were higher than $\frac{1}{2}A\frac{1}{2}M$ cross (81.9%), APRI line (80.4%) and Moshtohor line (80.9) rabbits, the percentages of dead sperms were also lower in V line (17.6%) and Sinai Gabali (17.9%) than Moshtohor line (19.1%), APRI line (19.6%) and $\frac{1}{2}A\frac{1}{2}M$ cross (18.1%) as shown in **Table 22**. The estimates of live sperms were lower than the percentages reported by **El-Tarabany et al. (2015)** in semen of Rex (96.3%), New Zealand White (94.6%), California (94.6%), and Gabali (94.2%) rabbits. **Anous et al. (2017)** recorded percentages of live sperms to be 93.1% in semen of New Zealand White bucks, 94.6% in Californian, 96.3% in Rex and 94.2% in Gabali.

The percentages of normal sperms for Sinai Gabali (87.5%) and $\frac{1}{2}A\frac{1}{2}M$ cross (87.3%) were higher than V line (86.6%), Moshtohor line (86.7) and APRI line (86.9%) and the percentages of abnormal sperm were also lower in Sinai Gabali and $\frac{1}{2}A\frac{1}{2}M$ cross than V line, Moshtohor line and APRI line rabbits (**Table 22**). **García-Tomás et al. (2006)** found that the percentages of normal sperms in C-line (85.2%) were lower than that of R-line (85.7%), C line x R line (86.2%) and R line x C line (88.6%). **Iraqi et al. (2012)** recorded sperm abnormality to be 88.3% in Egyptian Gabali and 87.1% in Moshtohor line.

The sperms cell concentration in semen (**Table 22**) showed that there were significant differences between Moshtohor line (468 x

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$\times 10^6/ml$), $\frac{1}{2}A\frac{1}{2}M$ cross ($465 \times 10^6/ml$), V line ($457 \times 10^6/ml$), APRI line ($423 \times 10^6/ml$) and Sinai Gabali ($409 \times 10^6/ml$) rabbits. Similarly, **Iraqi et al. (2012)** reported that the sperms cell concentration evidenced significant diversities among Sinai Gabali, V line and Moshtohor line ($405 \times 10^6/ml$, $474 \times 10^6/ml$ and $456 \times 10^6/ml$, respectively).

Table 22: Generalized least square means (GLM) and standard (SE) errors for semen traits as affected by genetic groups

Trait	Genetic group									
	V Line		M Line		A Line		$\frac{1}{2}A\frac{1}{2}M$		Sinai Gabali	
	GLM	SE	GLM	SE	GLM	SE	GLM	SE	GLM	SE
VE (ml)	0.64 ^c	0.01	0.69 ^a	0.01	0.61 ^d	0.01	0.68 ^b	0.02	0.54 ^c	0.03
pH	7.9 ^a	0.03	7.6 ^b	0.03	7.4 ^d	0.03	7.5 ^c	0.05	7.6 ^b	0.06
MS%	47.2 ^b	1.12	50.7 ^a	1.08	47.2 ^b	1.03	51.6 ^a	1.48	47.3 ^b	1.97
LS%	82.4 ^a	0.06	80.9 ^{ba}	0.60	80.4 ^b	0.57	81.9 ^{ba}	0.83	82.1 ^a	1.1
NS%	86.6 ^a	0.43	86.7 ^a	0.41	86.9 ^a	0.39	87.3 ^a	0.57	87.5 ^a	0.76
SCx10 ⁶	457 ^e	14.1	468 ^a	13.4	423 ^c	13.4	465 ^b	19.3	409 ^d	25.7
DS%	17.6 ^a	0.26	19.1 ^{ba}	0.60	19.6 ^b	0.57	18.1 ^{ba}	0.83	17.9 ^a	1.1
AS%	13.4 ^a	0.43	13.3 ^a	0.41	13.1 ^a	0.39	12.7 ^a	0.57	12.5 ^a	0.76

A=APRI-line, M=Moshtohor-line, Means within row, not sharing any letter are significantly different.

4.2.3 Heritabilities (h^2):

Heritabilities for semen traits estimated by Threshold model (TM) and VCE6 softwares were presented in **Table 23**. Heritabilities for semen traits estimated by TM software were very low and ranged from 0.01 to 0.03, while those estimated by VCE6 software were relatively moderate and ranged from 0.12 to 0.20. Accordingly, heritabilities estimated by VCE6 program are nearly similar to those obtained by **Khalil et al. (2007)** who reported different estimates of heritability for ejaculate volume, pH of semen, sperms concentration, sperms motility, abnormal sperms, and dead sperms to be 0.13, 0.12,

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0.12, 0.18, 0.16 and 0.17, respectively. The heritability of sperm motility estimated to be 0.16 to 0.18 compared to mass motility (Lavara et al., 2007). Similar to the estimates obtained by TM software, Lavara et al. (2013) reported low estimate of 0.05 for mass motility and Brun et al. (2009) estimated the heritability for ejaculate volume, sperms concentration and sperms production per ejaculate to be 0.13 ± 0.05 , 0.08 ± 0.04 and 0.07 ± 0.03 , respectively.

Table 23: Estimates of heritability (h^2) and standard errors (SE) for semen traits estimated by threshold (TM) and VCE6 softwares

Semen trait	($h^2 \pm$ SE estimated by VCE6)	($h^2 \pm$ SE estimated by TM)
Volume of ejaculate (ml)	0.14 ± 0.010	0.01 ± 0.01
pH of semen	0.14 ± 0.008	0.02 ± 0.01
Motility of sperms %	0.20 ± 0.014	0.03 ± 0.02
Live sperms %	0.12 ± 0.007	0.02 ± 0.01
Normal sperms %	0.17 ± 0.009	0.02 ± 0.01
Concentration of sperms $\times 10^6$	0.17 ± 0.010	0.01 ± 0.01
Dead sperms %	0.12 ± 0.007	0.02 ± 0.01
Abnormal sperms %	0.17 ± 0.009	0.02 ± 0.01

Number of records=1050

TM= Threshold Model; VCE6= Variance Component Estimation.

4.2.4 Crossbreeding analysis:

4.2.4.1 Direct additive effects (G^I):

The generalized least square solutions of G^I and their percentages for semen traits were given in **Table 24**. The estimable solutions of G^I were significantly in favour of Moshtohor line bucks by 0.04 ml, 0.2 %, 0.4 % and 20.5×10^6 /ml for volume of ejaculate, pH of semen, abnormal sperms and concentration of sperms, respectively ($p < 0.01$), with percentages ranged from 2.6 to 5.3 %, except for live sperms (0.4 %), normal sperms (0.4 %), motility of

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sperms (0.3 %) and dead sperms (0.05 %) were non-significant. **Khalil et al. (2007)** recorded that the estimates of G^I were 14.2 % for dead sperms, 12.4 % for concentration of sperms and 12.0 % for abnormal sperms in Saudi rabbits. **García-Tomás et al. (2006)** in crossing C line with R line rabbits found that G^I were 0.78 % for semen *pH*, 32.8 % for sperms concentration and 0.4 % for sperms mass motility.

Table 24: Generalized least square solutions and percentages of direct additive effects ($G^I = G_A^I - G_M^I$) and their standard errors (SE) for semen traits in crossing APRI and Moshtohor lines

Trait ⁺	G^I solution (units)	SE	G^I as% ⁺⁺
Volume of ejaculate (VE)	-0.04**	0.001	-5.3
<i>pH</i> of semen (<i>pH</i>)	-0.2**	0.003	-2.6
Motility of sperms % (MS)	-0.3 ^{NS}	0.1	-1.1
Live sperms % (LS)	-0.4 ^{NS}	0.04	-0.49
Normal sperms % (NS)	-0.4 ^{NS}	0.03	-0.5
Concentration of sperms $\times 10^6$ (SC)	-20.5**	0.9	-4.6
Dead sperms % (DS)	0.05 ^{NS}	0.04	0.3
Abnormal sperms % (AS)	0.4**	0.03	3.4

⁺ Number of records=695.

⁺⁺ Percentage computed as [Estimate of G^I in units / (A+M)/2] $\times 100$

NS= non-significant, **= $P \leq 0.01$.

4.2.4.2 Maternal effect (G^M):

The estimable solutions of G^M effects and their percentages for semen quality traits were given in **Table 25**. These estimable solutions for semen traits of volume of ejaculate, *pH* of semen, motility of sperms, dead sperms, abnormal sperms and concentration of sperms were significantly in favour of Moshtohor line by 0.05 *ml*, 0.4, 3.3 %, 1.2 %, 0.7 % and $22.8 \times 10^6/ml$, respectively, with

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percentages ranging from 1.2 to 8.1 %, except for live sperms (0.6 %) and normal sperms (1.3 %) were non-significant with percentages of 1.5 and 1.2 %, respectively. **Khalil et al. (2007)** showed that the differences in the maternal additive effects for volume of ejaculate (11.7%) were in favor of genes of the V line, while the estimates were in favor of Saudi genes for concentration of sperms (10.0%), percentage of abnormal sperms (13.2%), percentage of dead sperms (18.6%) and percentage of motility sperms (10.8%). **García-Tomás et al. (2006)** with two sire lines (C and R) observed that the differences between the two lines in maternal genetic effects were important and in favor of C line for ejaculate volume (14%), but in favor of R line for concentration of sperms (40%), total number of spermatozoa per ejaculate (23%), mass and individual motility and percentage of spermatozoa with presence of morphological abnormalities of neck-piece.

Table 25: Generalized least square solutions and percentages of maternal effects ($G^M = G^M_A - G^M_M$) and their standard errors (SE) for semen traits in crossing APRI and Moshtohor lines

Trait ⁺	G^M solution (units)	SE	G^M as% ⁺⁺
Volume of ejaculate <i>ml</i> (VE)	-0.05**	0.02	-8.1
<i>pH</i> of semen (<i>pH</i>)	-0.4**	0.002	-5.3
Motility of sperms % (MS)	-3.3*	0.07	-6.7
Live sperms % (LS)	-1.3 ^{NS}	0.03	-1.5
Normal sperms % (NS)	-0.6 ^{NS}	0.02	-1.2
Concentration of sperms $\times 10^6$ (SC)	-22.8**	0.8	-5.1
Dead sperms % (DS)	1.2**	0.03	6.3
Abnormal sperms % (AS)	0.7**	0.001	4.9

⁺ Number of records=695.

⁺⁺Percentage computed as [Estimate of G^M in units/ (A+M)/2] x100

NS= non-significant, *= $P \leq 0.05$ and **= $P \leq 0.01$.

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4.2.4.3 Direct heterosis (H^I):

The generalized least square solutions of H^I and their percentages for semen traits were presented in **Table 26**. The estimable solutions of direct heterosis for semen quality in terms of ejaculate volume, *pH* of semen, motility of sperms, live sperms, dead sperms, abnormal sperms and concentration of sperms were significantly associated with improvements in these traits by 0.09 *ml*, 0.7, 5.8 %, 3.1 %, 3.3 %, 1.1 % and $30.7 \times 10^6/ml$ and the percentages ranged from 1.1 to 16.9 %.

Table 26: Generalized least square solutions and percentages of heterotic effects solutions ($H^I = H^I_A - H^I_M$) and their standard errors (SE) for semen traits in crossing APRI and Moshtohor line

Trait ⁺	H^I solution (units)	SE	H^I as % ⁺⁺
Volume of ejaculate <i>ml</i> (VE)	0.09**	0.002	13.3
<i>pH</i> of semen (<i>pH</i>)	0.7**	0.003	9.4
Motility of sperms % (MS)	5.8**	0.1	11.9
Live sperms % (LS)	3.1**	0.06	3.8
Normal sperms %	1.01 ^{NS}	0.04	1.3
Concentration of sperms $\times 10^6$ (SC)	30.7**	0.9	6.9
Dead sperms % (DS)	-3.3**	0.06	-16.9
Abnormal sperms % (AS)	-1.1**	0.04	-8.01

⁺ Number of records=695.

⁺⁺Percentage computed as [Estimate of H^I in units/ (A+M)/2] x100

NS= non-significant, **= $P \leq 0.01$.

In Saudi Arabia, **Al-Sobayil And Khalil (2002)** estimated moderate direct heterosis to be 21.5% for percentages of abnormal sperms ($P < 0.05$) and 20.3% for dead sperms ($P < 0.05$). **Khalil et al. (2007)** indicated that crossbred bucks were associated with significant heterotic effects in ejaculate volume (11.6%), sperms concentration (10.5%), percentages of motile sperms (9.8%) along

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with a reduction in percentages of abnormal sperms (10.8%) and dead sperms (23.5%). **El-Tarabany et al. (2015)** in crossbreeding experiment between New Zealand White and Flander found that the estimates of direct heterosis for ejaculate volume, mass motility, individual motility and sperms cell concentration were positive and significant.

4.3 Molecular analyses of *GH* gene and its association with growth and semen traits:

4.3.1 Allele and genotype frequencies of *GH* gene in each genetic group:

PCR products of 231 bp and PCR-RFLP patterns of *GH* gene were detected in the studied genetic groups (**Figure 1A and B**). Three genotypes TT, CC and TC were detected. The allelic and genotypic frequencies of the *GH* polymorphisms estimated across the five populations are shown in **Table 27**.

Table 27: Genotypic and allelic frequencies of the *GH* gene in different genetic groups studied

Genetic group	N	Genotype frequency			Allele frequency	
		TT	TC	CC	T	C
V line	27	0.48 ^a	0.45 ^c	0.07 ^b	0.70 ^a	0.30 ^d
M line	19	0.32 ^c	0.47 ^d	0.21 ^a	0.55 ^d	0.45 ^a
A line	26	0.31 ^c	0.62 ^b	0.07 ^b	0.62 ^c	0.38 ^b
½A½M	21	0.28 ^d	0.67 ^a	0.04 ^c	0.62 ^c	0.38 ^b
Gabali	18	0.39 ^b	0.56 ^c	0.05 ^c	0.68 ^b	0.32 ^c

A=APRI-line and M=Moshtohor-line, The estimate with the same letters in each column are not significantly different ($p \leq 0.05$), SE= standard error.

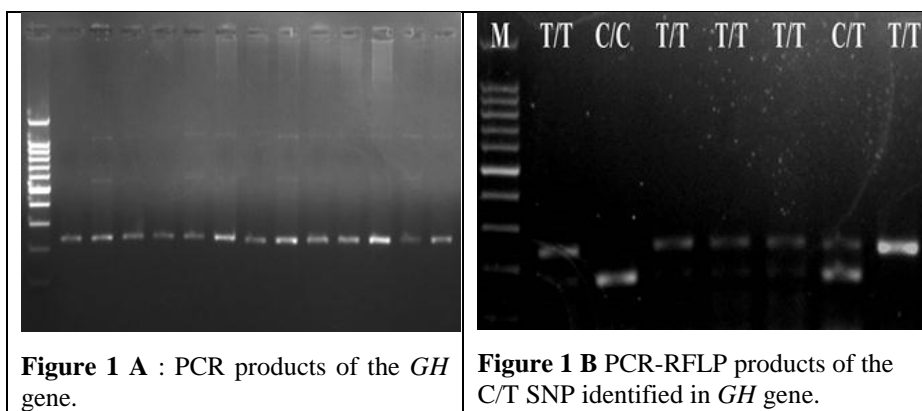
Across different genetic groups, the frequency of TT genotype was highly significant ($P < 0.05$) and ranged from 0.28

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in $\frac{1}{2}A\frac{1}{2}M$ to 0.48 in V line rabbits. For CC genotype, the highest and significant ($P < 0.05$) frequency was recorded in Moshtohor line (0.21) and the lowest frequency was recorded in $\frac{1}{2}A\frac{1}{2}M$ rabbits (0.04). On contrast, the highest and significant ($P < 0.05$) frequency of TC genotype was recorded in $\frac{1}{2}A\frac{1}{2}M$ (0.67) and the lowest frequency in V line (0.45). The allelic frequency showed the same trend as the genotypic frequency, where the highest frequency for C allele was recorded by Moshtohor line (0.45) and the lowest frequency in was Gabali (0.32). **Fontanesi et al. (2008)** reported the frequencies of C and T alleles at c.747+34 C>T SNP of *GH* gene to be 0.51 and 0.49, respectively. In the commercial paternal line rabbit population, **Rafayová et al. (2009)** displayed frequencies of 0.67 and 0.33, for T and C alleles at c.747+34 C>T in M91 and P91 rabbit lines, respectively. The frequencies of T and C alleles were reported by **Markowska et al. (2010)** to be 0.38 and 0.62 in Polish and White Flemish Giants rabbits, respectively and 0.43 and 0.57 by **Bindu et al. (2011)** in a pooled population of New Zealand White and Soviet Chinchilla and their crosses. **Amalianingsih and Brahmantiyo (2014)** found that the allele frequency in New Zealand White and Californian rabbits was 0.625 and 0.375, respectively. **Hussein et al. (2015)** reported that the observed frequencies were 0.540 for allele T and 0.460 for allele C in 202 APRI rabbits. In the synthetic inbred F1 and F2 of New Zealand White, the frequency of the heterozygous genotype CT was 0.696 and 0.609, respectively, while the frequency of the homozygous CC genotype was the lowest (0.043 and 0.000), and the respective frequencies of the homozygous TT genotype were 0.261 and 0.391. This presumed a preponderance of the T allele

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(0.609 and 0.696) over the C allele (0.391 and 0.304) in these groups. **Hristova et al. (2018)** in outbred rabbits reported that the allelic frequencies were 0.613 for allele C and 0.387 for allele T and the frequency of the homozygous CC genotype was higher than that of the homozygous TT genotype (0.300 vs. 0.075).



The effective numbers of alleles (N_e) and Chi-square values within each genetic group were presented in **Table 28**. The highest allelic numbers of N_e was obtained for Moshtohor line (1.978), while the lowest allelic numbers were obtained in V line (1.715) and Gabali breed (1.800). Chi-Square tests showed that the deviation from Hardy-Weinberg equilibrium were not significant and ranged from 0.034 to 3.59 in all studied populations. Similar results were obtained by **Abdel-Kafy et al. (2016)** who found that the chi-square value was not significant for APRI population.

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Table 28: The effective numbers of alleles (N_e) and Chi-square values for Hardy-Weinberg equilibrium (HWE) characterizing the *GH* gene in different genetic groups studied

Genetic group	N	N_e	P-value	χ^2 value for HWE
V line	27	1.715 ^d	0.732	0.117 ^{ns}
M line	19	1.978 ^a	0.855	0.034 ^{ns}
A line	26	1.899 ^b	0.126	2.340 ^{ns}
½A½M	21	1.893 ^b	0.058	3.590 ^{ns}
Gabali	18	1.800 ^c	0.289	1.125 ^{ns}
Overall mean ± SE		1.857±0.045		

A=APRI line, M=Moshtohor line. The estimate with the same letters in each column are not significantly different ($p \leq 0.05$); χ^2 = Chi-square value Hardy-Weinberg equilibrium; ns= Non-significant.

4.3.2 The heterozygosity and polymorphic information content (PIC) in each genetic group:

The values of observed (H_o) and expected (H_e) heterozygosities, polymorphic information content (PIC) and the reduction in heterozygosity due to inbreeding (F_{IS}) for *GH* gene of were presented in **Table 29**. The levels of genetic diversity across the five studied populations were intermediate ($H_o = 0.551$, $H_e = 0.471$, $PIC = 0.358$ and $F_{IS} = -0.198$). However, the observed heterozygosity was higher than the expected heterozygosity in all genetic groups and the values of observed heterozygosity ranged from 0.444 in V line to 0.667 in ½A½M. While the values of expected heterozygosity were ranged from 0.425 in V line to 0.508 in Moshtohor line.

According to the classification of PIC values for *GH* gene ($PIC < 0.25$ = low polymorphism; $0.25 < PIC < 0.50$ = intermediate polymorphism; $PIC > 0.50$ = high polymorphism), the values of PIC showed moderate level ($0.25 < PIC < 0.50$) and ranged from 0.332 in

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V line to 0.375 in M line. **Amalianingsih and Brahantiyo (2014)** reported similar *PIC* values for Rex, Satin and Reza rabbit, being 0.207, 0.375 and 0.373, respectively. The variation between *PIC* values obtained here might be due to the existence of the potential population dynamics, selection program and the nature of the sampling process. This means that C/T SNP of *GH* gene had high degree of genetic information in rabbit's populations.

Table 29: The observed (*Ho*) and expected (*He*) heterozygosities, the polymorphic information content (*PIC*) and reduction in heterozygosity due to inbreeding (*F_{IS}*) characterizing *GH* gene in different genetic groups

Genetic Group	N	<i>Ho</i>	<i>He</i>	<i>PIC</i>	<i>F_{IS}</i>
V line	27	0.444 ^c	0.425 ^d	0.332 ^c	-0.066
M line	19	0.474 ^d	0.508 ^a	0.375 ^a	0.042
A line	26	0.615 ^b	0.483 ^b	0.360 ^{ba}	-0.300
½A½M	21	0.667 ^a	0.483 ^b	0.360 ^{ba}	-0.413
Gabali	18	0.556 ^c	0.457 ^c	0.341 ^{bc}	-0.250
Overall mean ± SE		0.551 ±0.014	0.471 ±0.042	0.358 ±0.006	-0.198 ±0.082

A=APRI line and M=Moshtohor line; The estimate with the same letters in each column are not significantly different ($p \leq 0.05$); SE= standard error; *F_{IS}*= reduction in heterozygosity due to inbreeding within each breed.

The reductions in heterozygosity for each locus across the five investigated genetic groups were moderate to low as shown in **Table 29**. The highest *F_{IS}* was observed in Moshtohor line (0.042) and the lowest value was observed in ½A½M (-0.413). The maintenance of high heterozygosity in heterogeneous populations, despite the presence of narrow inbreeding, allowed expecting a weaker negative effect of inbreeding depression on members of such populations (**Tanchev, 2015**). Our results were in agreement with **Hristova et al.**

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(2018) who recorded higher observed heterozygosity than the expected ones for *GH* gene, and therefore a negative inbreeding coefficients ($F_{IS} = -0.317$ for outbred NZW rabbits, -0.460 for inbred F1 and -0.438 for inbred F2) were obtained.

4.3.3 Polymorphic associations between genotypes of *GH* gene and growth traits:

The generalized least square means for SNP genotypes of *GH* gene in V line, M line, A line and $\frac{1}{2}A\frac{1}{2}M$ cross have shown significant effects on most body weights and daily weight gains (Table 30). Similarly, several studies reported significant associations between *GH* gene and body weights in rabbits (e.g. Fontanesi et al., 2008; Abdel-Kafy et al., 2016; El-Sabrout and Aggag, 2017).

In V line rabbits, CC genotype was absent and the association of TT and TC genotypes of *GH* gene was significant ($P < 0.05$), since TT genotype was heavier for body weight at 4 weeks than TC genotype (Table 30). However, TT genotype was lower than TC genotype for body weights at 6, 8, 10 and 12 weeks. The TC genotype was positively associated with an increase in body weights at 6, 8, 10 and 12 weeks of age by 32, 9, 57 and 108 g, respectively. For daily gain, the CC genotype was absent and the TT genotype was lower than TC genotype and the difference between SNP genotypes of *GH* gene were significant ($P < 0.05$) for most body gains studied (Table 30). These data were in agreement with El-Aksher et al. (2016) who found significant associations ($P < 0.05$) between the genotypes SNPs of progesterone receptor gene (*PGR* gene) and body

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weights for V line and Sinai Gabali rabbits. Also, **El-Sabrout and Aggag (2017)** found significant ($P<0.05$) associations between growth hormone, insulin-like growth factor 1, myostatin, melanocortin 4 receptor, growth hormone receptor and phosphoglycerate mutase genes and body weights in V line and Alexandria rabbits.

In Moshtohor line rabbits, the differences among genotypes of *GH* gene were significant ($P<0.05$) and the TC genotype was heavier in body weights at 4, 6, 8, 10 and 12 weeks than CC and TT genotypes (**Table 30**). The TC genotype was positively associated with an increase in body weights at 4, 8, 10 and 12 weeks of age by 134, 119, 636 and 734 g, respectively. The differences among genotypes of *GH* gene in all daily weight gains were significant ($P<0.05$) and the TT genotype was lower in daily weight gains at 6-8, 8-10 and 10-12 weeks than CC and TC genotypes. The TT genotype was heavier in daily gain at 4-6 weeks than CC and TC genotypes. **Lu et al. (2011) and Peng et al. (2013)** reported significant association between myostatin gene (*MSTN*) and growth traits in different genetic groups of rabbits.

The differences between genotypes of *GH* gene in APRI line rabbits were significant ($P<0.05$) and CC genotype was heavier in body weight at 4 and 10 weeks than TT and TC genotypes, while the TC genotype was heavier in body weights at 6, 8, and 12 weeks than CC and TT genotypes (**Table 30**). The TC genotype was positively associated with an increase in body weights at 6, 8 and 12 weeks of age by 43, 61 and 305 g, respectively. Significant differences ($P<0.05$) were recorded between genotypes of *GH* gene in daily

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weight gains at 6-8 and 10-12 weeks in APRI line. In China, **Zhang et al. (2012)** found significant associations ($P<0.05$) between growth hormone receptor gene (*GHR*) and body weights in Tianfu Black, Ira and Champagne rabbits. Additionally, **Wu et al. (2015)** reported significant association ($P<0.05$) between phosphoglycerate mutase gene (*PGAM2*) and growth traits in the same Chinese rabbit breeds.

The differences between genotypes of *GH* gene in $\frac{1}{2}A\frac{1}{2}M$ cross rabbits were significant ($P<0.05$) in all body weights except body weight at 4 weeks and the CC genotype was absent (**Table 30**). The TT genotype was heavier than TC genotype in all body weights and this TT genotype was positively associated with an increase of 22, 6, 88 and 29 g in body weights at 6, 8, 10 and 12 weeks of age, respectively. For body gains, the differences between genotypes of *GH* gene in $\frac{1}{2}A\frac{1}{2}M$ rabbits were significant ($P<0.05$) and the TT genotype was heavier than TC genotype in all body gains, and the CC genotype was absent, while the TC genotype was higher than TT genotype at 8-10 weeks. **Abdel-Kafy et al. (2016)** studied the association between Myostatin (MSTN) gene and growth traits in APRI line and found that allele T at the c.747+34C>T SNP was significantly associated ($P<0.05$) with an increase in body weight at 12 weeks of age. Moreover, **Othman et al. (2015)** found that the genotypes of fibroblast growth factor 5 gene (*FGF-5*) were associated significantly ($P<0.05$) with body weights in different populations of rabbits in Egypt.

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Table 30: Generalized least square means and their standard errors (GLM±SE) for body weights (BW) and daily weight gains (DG) as affected by SNP genotypes of *GH* gene in each genetic group separately

Trait	Genetic group	Number of records	Genotypes					
			TT		TC		CC	
			GLM	SE	GLM	SE	GLM	SE
BW4	V line	62	499 ^a	11.6	456 ^b	12.9	--	--
	M line	46	450 ^c	31.6	584 ^a	22.7	498 ^b	30.8
	A line	35	495 ^a	19.4	474 ^b	8.8	496 ^a	21.2
	½A½M	68	494 ^a	11.7	495 ^a	7.4	--	--
BW6	V line	61	770 ^b	19.9	802 ^a	21.5	--	--
	M line	46	795 ^b	30.8	796 ^b	22.3	827 ^a	30.2
	A line	34	707 ^b	26.2	737 ^a	10.9	694 ^c	25.
	½A½M	64	780 ^a	19.2	758 ^b	12.3	--	--
BW8	V line	58	1143 ^b	22.0	1152 ^a	23.9	--	--
	M line	46	1029 ^c	43.4	1148 ^a	31.4	1098 ^b	42.7
	A line	34	1093 ^a	70.6	1095 ^a	28.8	1034 ^c	69.8
	½A½M	60	1043 ^a	24.7	1037 ^b	16.1	--	--
BW10	V line	55	1297 ^b	15.9	1354 ^a	16.9	--	--
	M line	46	1071 ^c	189	1707 ^a	134	1288 ^b	164
	A line	34	1361 ^b	66.2	1271 ^c	27	1399 ^a	65.4
	½A½M	60	1349 ^a	26.9	1261 ^b	17.8	--	--
BW12	V line	50	1696 ^b	24.5	1804 ^a	24.5	--	--
	M line	45	1240 ^c	75	1974 ^a	52.1	1564 ^b	70.8
	A line	29	1673 ^b	94.8	1917 ^a	32.1	1612 ^c	89.1
	½A½M	59	1686 ^a	31.7	1657 ^b	20.7	--	--
DG4-6	V line	61	18.8 ^b	1.29	25.3 ^a	1.35	--	--
	M line	46	24.6 ^a	2.9	15.1 ^c	2.05	23.5 ^b	2.5
	A line	34	14.7 ^b	1.65	18.6 ^a	0.71	14.1 ^b	1.42
	½A½M	64	20.3 ^a	1.36	18.9 ^b	0.94	--	--
DG6-8	V line	58	28.2 ^a	1.55	20.4 ^b	1.63	--	--
	M line	46	16.7 ^c	3.6	25.1 ^a	2.5	19.3 ^b	3.1
	A line	34	27.3 ^a	4.64	20.1 ^b	2.02	18.8 ^c	4.04
	½A½M	60	18.9 ^b	1.62	20.2 ^a	1.10	--	--
DG8-10	V line	55	9.3 ^b	1.27	20.7 ^a	1.37	--	--
	M line	46	22.2 ^b	13.4	11.3 ^c	10.4	32.8 ^a	12.4
	A line	34	19.3 ^b	2.79	18.1 ^c	1.21	31.4 ^a	2.42
	½A½M	60	16.8 ^b	1.62	22.1 ^a	1.09	--	--
DG10-12	V line	50	29.6 ^a	1.43	21.3 ^b	1.40	--	--
	M line	45	19.3 ^c	3.02	25.5 ^b	2.23	26.9 ^a	2.80
	A line	29	22.6 ^c	5.52	24.0 ^b	1.95	34.9 ^a	4.51
	½A½M	59	31.1 ^a	1.82	22.5 ^b	1.25	--	--

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GH=Growth hormone gene; A=APRI line, M=Moshtohor line; letters in the same row indicate significant differences at $P<0.05$.

4.3.4 Polymorphic associations between genotypes of *GH* gene and semen traits:

The generalized least square means for SNP genotypes of *GH* gene in V line, Sinai Gabali, M line, A line and $\frac{1}{2}A\frac{1}{2}M$ cross showed significant effects on most semen traits (**Table 31**). The TC genotype was the highest in volume of ejaculate, motility of sperms, normal sperms and live sperms, along with the lowest abnormal and dead sperms, while the CC genotype was the highest in concentration of sperms and *pH* of semen. These results agree with **Nikbin et al. (2018)** who detected the polymorphic associations of four SNPs in caprine FSH β and LH β with semen quality traits of male goats and reported also significant associations of the candidate genes with semen quality traits in terms of progressive motility, and abnormality of fresh semen ($P < 0.05$), motility, velocity, and viability traits of post-thaw semen ($P < 0.05$).

In V line rabbits, the associations of TT, TC and CC genotypes of *GH* gene with some semen traits of ejaculate volume, normal sperms and concentration of sperms were significant ($P < 0.05$) and TT genotype showed higher values than CC and TC genotypes with an increase in semen traits of ejaculate volume, normal sperms and concentration of sperms by 0.14 ml, 2.2 % and $45 \times 10^6/ml$, respectively (**Table 31**). While the TC genotype was higher than TT and CC genotypes for *pH* of semen, motility of sperms and live sperms with an increase to be 0.2, 3.5 % and 3.3 %, respectively, expect the TT genotype was the lowest in dead sperms

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and abnormal sperms and with a decrease to be 3.1 % and 1.9 %, respectively (**Table 31**).

In Moshtohor line rabbits, the differences among TT, TC and CC genotypes of *GH* gene in most semen traits were significant (**Table 31**). The TC genotype was the highest in *pH* of semen, motility of sperms, live sperms, normal sperms and concentration of sperms with an increase of 2, 3.1 %, 2.5 %, 1.7 % and $20.8 \times 10^6/ml$, respectively, while the CC genotype was the highest in volume of ejaculate with an increase of 2 *ml*, except the TT genotype was the lowest in dead sperms and abnormal sperms with a decrease of 1.8 % and 2.5 %, respectively.

In APRI line rabbits, the differences among TT, TC and CC genotypes of *GH* gene were significant for all semen traits and TT genotype was the lowest in *pH* of semen, motility of sperms, concentration of sperms and dead sperms (**Table 31**). The CC genotype was the highest in volume of ejaculate, *pH* of semen and concentration of sperms with an increase of 1.3 *ml*, 2.0 and $232.2 \times 10^6/ml$, respectively and TC genotype was the highest in motility of sperms, live sperms and normal sperms, with an increase of 2.1 %, 2.3 % and 2.7 %, respectively. The TT genotype was the lowest in dead sperms and abnormal sperms with a decrease of 5 % and 5.2 %, respectively.

In $\frac{1}{2}A\frac{1}{2}M$ cross rabbits, the differences among TT, TC and CC genotypes of *GH* gene were significant in all semen traits (**Table 31**). The TT genotype was the highest in volume of ejaculate, *pH* of semen, motility of sperms, live sperms, normal sperms and concentration of sperms, with an increase of 0.1 *ml*, 0.2, 3.6 %, 4.5

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%, 1.0 % and $97.9 \times 10^6/ml$, respectively, while the CC genotype was the lowest in dead sperms and abnormal sperms with a decrease of 4.3 % and 1.0 %, respectively.

In Sinai Gabali rabbits, the differences among TT, TC and CC genotypes of *GH* gene were significant in all semen traits (**Table 31**). The TT genotype was the highest in *pH* of semen, motility of sperms, normal sperms and concentration of sperms, with an increase of 0.1, 8.7 %, 0.7 % and $95.1 \times 10^6/ml$, respectively. The TC genotype was the highest in volume of ejaculate, live sperms, with an increase of 0.24 *ml* and 2.6%, respectively, while the CC genotype was the lowest in dead sperms and abnormal sperms, with a decrease of 2.5% and 0.6%, respectively.

Table 33: Generalized least square means (GLM) and their standard errors (SE) for semen traits as affected by SNP genotypes of *GH* gene in each genetic group separately

Trait	Genetic group ⁺	Genotypes					
		TT		TC		CC	
		GLM	SE	GLM	SE	GLM	SE
Volume of ejaculate, <i>ml</i> (VE)	V line	0.28 ^a	0.02	0.25 ^a	0.03	0.14 ^b	0.05
	M line	0.1 ^c	0.03	1.1 ^b	0.03	2.1 ^a	0.03
	A line	1.9 ^c	0.04	2 ^b	0.03	3.2 ^a	0.09
	½A½M	0.7 ^a	0.04	0.6 ^a	0.04	0.7 ^a	0.09
	G	0.39 ^b	0.04	0.47 ^a	0.03	0.23 ^c	0.10
<i>pH</i> of semen, (<i>pH</i>)	V line	7.4 ^a	0.06	7.6 ^a	0.06	7.4 ^a	0.13
	M line	6.9 ^c	0.06	8.9 ^a	0.06	7.9 ^b	0.10
	A line	8.8 ^b	0.06	8.9 ^b	0.04	10.8 ^a	0.15
	½A½M	7.4 ^a	0.09	7.4 ^a	0.09	7.2 ^a	0.19
	G	7.6 ^a	0.07	7.5 ^a	0.06	7.5 ^a	0.19
Motility of sperms, % (MS)	V line	46.5 ^b	1.7	48.6 ^a	1.8	45.1 ^c	3.7
	M line	51.9 ^b	1.8	55 ^a	1.7	53.1 ^b	2.9
	A line	45.9	2.0	48	1.4	47.5	4.8
	½A½M	50.7 ^a	2.4	50.4 ^a	2.3	47.1 ^b	5.0
	G	49.2 ^a	2.7	40.5 ^c	2.2	44.4 ^b	7.1
Dead sperms, % (DS)	V line	16.2 ^c	0.98	17.7 ^b	1.02	19.3 ^a	2.11
	M line	17.1 ^b	1.02	18.9 ^a	0.99	17.1 ^b	1.69

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	A line	21.6 ^c	1.15	23 ^b	0.82	26.6 ^a	2.75
	½A½M	19.4 ^a	1.60	18.2 ^a	1.52	15.1 ^b	3.37
	G	20.9 ^a	1.12	18.9 ^b	0.94	18.4 ^b	2.96
Live sperms, % (LS)	V line	81.4 ^a	0.99	82.8 ^a	1.02	79.5 ^b	2.11
	M line	81.4 ^b	1.03	83.9 ^a	0.98	83.6 ^a	1.69
	A line	79.8 ^b	1.15	81.1 ^a	0.82	78.8 ^c	2.74
	½A½M	84.7 ^a	1.61	81.7 ^b	1.52	80.5 ^b	3.38
	G	78.8 ^b	1.11	81.4 ^a	0.93	80.7 ^a	2.96
Normal sperms, % (NS)	V line	85.7 ^a	0.71	84.7 ^b	0.73	83.5 ^c	1.52
	M line	87.2 ^b	0.88	88.9 ^a	0.84	88.1 ^a	1.45
	A line	85.3 ^b	0.59	86.8 ^a	0.42	84.1 ^c	1.41
	½A½M	87.3 ^a	0.82	87.1 ^a	0.77	86.3 ^b	1.73
	G	87.5 ^a	0.82	86.8 ^b	0.68	87.4 ^a	2.16
Abnormal sperms, % (AS)	V line	13.4 ^c	0.72	14.4 ^b	0.74	15.3 ^a	1.52
	M line	11.3 ^c	0.88	13.8 ^a	0.84	12.6 ^b	1.45
	A line	16.1 ^b	0.59	17.5 ^b	0.42	21.3 ^a	1.41
	½A½M	13.5 ^a	0.82	12.8 ^a	0.77	12.5 ^a	1.72
	G	12.3 ^a	0.82	12.9 ^a	0.68	12.3 ^a	2.16
Concentration of sperms, X10 ⁶ /ml (SC)	V line	468 ^a	0.02	453 ^b	0.02	423 ^c	0.04
	M line	445 ^b	0.03	462 ^a	0.02	442 ^b	0.04
	A line	501 ^b	0.03	527 ^b	0.02	734 ^a	0.07
	½A½M	487 ^a	0.04	421 ^b	0.04	389 ^c	0.09
	G	426 ^a	0.05	331 ^b	0.04	336 ^b	0.13

⁺ A=APRI line, M=Moshtohor line, G= Sinai Gabali. Number of records = 275, 239, 312, 144 and 80 for V line, M line, A line, ½A½M and Sinai Gabali, respectively.

Letters in the same row indicate significant differences at $P<0.05$.

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A simple crossbreeding experiment was conducted between males of APRI line (A) and females of Moshtohor line (M) rabbits. These two lines are new synthetic lines raised in Egypt for meat production. APRI line was formed in the experimental stations belonging to the Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt, while Moshtohor line was established in the Faculty of Agriculture, Benha University, Egypt. The crossbreeding scheme was performed during two consecutive production seasons (2015/2016 and 2016/2017) at the rabbitry of the Animal Production Department, Faculty of Agriculture, Benha University. The molecular analyses were conducted at the Central Laboratory, Faculty of Veterinary Medicine, Benha University. The main objectives of the present study were: (1) to estimate variance components, heritability, direct additive, maternal additive, and direct heterotic effects for post-weaning body weights at (4, 6, 8, 10 and 12 weeks of age) and daily weight gains (during the intervals 4-6, 6-8, 8-10 and 10-12 weeks) and semen quality traits (ejaculate volume, *pH* semen, sperms motility, sperms concentration, live and dead sperms, normal and abnormal sperms), (2) to use the PCR-RFLP technique to genotype the C>T SNP located in the promoter region of *GH* gene in five genetic groups of rabbits (APRI, Moshtohor, $\frac{1}{2}A\frac{1}{2}M$, V line and Sinai Gabali) and (3) to detect the polymorphic associations between (C>T) SNP genotypes of *GH* gene and growth or semen quality traits. A total number of 1201 weaned rabbits fathered by 179 sires and mothered by 261 dams

were used in quantitative and molecular analyses of growth traits, while a total of 1050 ejaculates collected from 149 bucks were used in semen quality traits. For detecting the associations between genotypes of *GH* gene and growth or semen traits, the method of Generalized Least Squares (GLS) was used. The most relevant results of this study could be summarized as follows:

Quantitative genetic analyses for growth and semen quality traits:

- Estimates of heritability for body weights and gains were mostly low or moderate and ranging from 0.06 to 0.18 for estimates obtained from VCE software and 0.09 to 0.18 for estimates obtained from TM software. The estimates for semen quality traits resulted from TM software were very low and ranged from 0.01 to 0.03, while those estimated by VCE6 software were relatively moderate and ranged from 0.12 to 0.20.
- The generalized least square solutions of direct additive effects (G^I) were significantly ($P<0.01$) in favor of Moshtohor line rabbits by 13.5 to 48.1 g for body weights and 0.5 to 3.7 g for daily gains, with G^I percentages ranging from 1.4 to 4.3 % for body weights and 2.1 to 18.6 % for daily gains. For semen quality traits, the solutions of G^I were significantly ($P<0.01$) in favor of Moshtohor line by 0.04 ml, 0.2, 0.4% and $20.5 \times 10^6/ml$ for volume of ejaculate, pH of semen, abnormal sperms and sperms concentration, respectively, with percentages ranging from 2.6 to 5.3 %.
- The generalized least square solutions of maternal additive effect (G^M) were significantly in favor of Moshtohor line by 14.2 to

51.8 g for body weights and 0.48 to 3.1 g for daily weight gains in weight, with G^M percentages ranging from 2.8 to 5.4 % for body weights and 1.8 to 15.0% for daily gains. For semen traits, the solutions of G^M for ejaculate volume, pH of semen, motility of sperms, dead sperms, abnormal sperms and sperms concentration were also significantly in favor of Moshtohor line by 0.05 ml, 0.4, 3.3%, 1.2%, 0.7% and $22.8 \times 10^6/ml$, respectively, with percentages ranging from 1.2 to 8.1 %.

- The generalized least square solutions of direct heterosis (H^I) were positive and highly significant and ranged from 15.5 to 87.1 g for body weights and from 1.6 to 2.79 g for daily gains, with H^I percentages ranging from 3.1 to 8.2 % for body weights and 1.1 to 13.0 % for daily gains. For semen quality traits, the solutions of H^I for ejaculate volume, pH of semen, motility of sperms, live sperms, dead sperms, abnormal sperms and concentration of sperms were significantly associated with improvements in these traits by 0.09 ml, 0.7, 5.8%, 3.1%, 3.3%, 1.1% and $30.7 \times 10^6/ml$, with heterotic percentages ranging from 3.8 to 16.9% .

Molecular characterization of growth hormone gene in different genetic groups

- Across all genetic groups, the frequency of TT genotype of GH gene was highly significant ($P < 0.05$) and ranged from 0.48 in V line followed by 0.39 in Gabali, 0.32 in M line, 0.31 in A line and 0.28 in $\frac{1}{2}A\frac{1}{2}M$ rabbits. For CC genotype, the highest and significant ($P < 0.05$) frequency was recorded in Moshtohor line (0.21) and the lowest frequency was recorded in $\frac{1}{2}A\frac{1}{2}M$ rabbits

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(0.04), while in TC genotype the highest and significant frequency ($P < 0.05$) was recorded in $\frac{1}{2}A\frac{1}{2}M$ (0.67) and the lowest frequency in V line (0.45). The highest frequency for C allele was recorded by Moshtohor line (0.45) and the lowest frequency was in Gabali (0.32).

- For (T/C) SNP of *GH* gene, the highest effective numbers of alleles (N_e) were obtained in Moshtohor line (1.978), followed by APRI line (1.899) then $\frac{1}{2}A\frac{1}{2}M$ cross (1.893), while the lowest allelic numbers were obtained in V line (1.715) and Gabali breed (1.800).
- The deviations from Hardy-Weinberg equilibrium were not significant in all populations studied.
- The observed heterozygosity (H_o) for *GH* gene was higher than the expected heterozygosity (H_e) in all genetic groups and the values of expected heterozygosity ranged from 0.444 in V line to 0.667 in $\frac{1}{2}A\frac{1}{2}M$ cross, while the values of the observed heterozygosity ranged from 0.425 in V line to 0.508 in Moshtohor line.
- All the values of polymorphic information content (PIC) were moderate; being 0.332, 0.375, 0.360, 0.360 and 0.341 in V line, M line, APRI line, $\frac{1}{2}A\frac{1}{2}M$ and Gabali, respectively.
- The reduction in heterozygosity (F_{IS}) for each locus across the five investigated genetic groups were moderate or low where the highest F_{IS} was observed in Moshtohor line (0.042) and the lowest value was observed in $\frac{1}{2}A\frac{1}{2}M$ (-0.413).

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- The levels of genetic diversity across the five studied populations were intermediate ($H_o = 0.551$, $H_e = 0.471$, $PIC = 0.358$ and $F_{IS} = -0.198$).

Molecular associations among genotypes of *GH* gene and growth traits

- In V- line rabbits, CC genotype was absent and the association of TT and TC genotypes of *GH* gene was significant ($P < 0.05$) with growth traits, since TT genotype was lower than TC genotype for body weights at 6, 8, 10 and 12 weeks, i.e. TC genotype was positively associated with an increase in body weights at 6, 8, 10 and 12 weeks of age by 32, 9, 57 and 108 g, respectively. For daily gain, the CC genotype was absent and the TT genotype was also lower than TC genotype and the differences between SNP genotypes of *GH* gene were significant ($P < 0.05$) for most daily gains.
- In Moshtohor line rabbits, the differences among genotypes of *GH* gene were significant ($P < 0.05$) and TC genotype was heavier in body weights at 4, 6, 8, 10 and 12 weeks than CC and TT genotypes. The TC genotype was positively associated with an increase in body weights at 4, 8, 10 and 12 weeks of age by 134, 119, 636 and 734g, respectively. The differences among genotypes of *GH* gene in all daily weight gains were significant ($P < 0.05$) and TT genotype was lower in most daily weight gains at 6-8, 8-10 and 10-12 weeks than CC and TC genotypes.
- The differences between genotypes of *GH* gene in APRI line rabbits were significant ($P < 0.05$) and CC genotype was heavier

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in body weights at 4 and 10 weeks than TT and TC genotypes, while TC genotype was heavier in body weights at 6, 8, and 12 weeks than CC and TT genotypes. The TC genotype was positively associated with an increase in body weights at 6, 8 and 12 weeks of age by 43, 61 and 305 g, respectively. Significant differences ($P<0.05$) were recorded between genotypes of *GH* gene in daily weight gains at 6-8 and 10-12 weeks.

- The differences between genotypes of *GH* gene in $\frac{1}{2}A\frac{1}{2}M$ cross were significant ($P<0.05$) in all body weights except body weight at 4 weeks where CC genotype was absent. The TT genotype was heavier than TC genotype in all body weights and this TT genotype was positively associated with an increase of 22, 6, 88 and 29 g in body weights at 6, 8, 10 and 12 weeks of age, respectively. For body gains, the differences between genotypes of *GH* gene in $\frac{1}{2}A\frac{1}{2}M$ rabbits were significant ($P<0.05$) and TT genotype was higher in all body gains than TC genotype, and the CC genotype was absent, while TC genotype was higher in 8-10 weeks body gains than TT genotype.

Molecular associations among genotypes of *GH* gene and semen quality traits

- In V line rabbits, the associations of TT, TC and CC genotypes of *GH* gene with some semen traits of volume of ejaculate, normal sperm and concentration of sperm were significant ($P < 0.05$) and TT genotype showed higher values than CC and TC genotypes with an increase in semen traits of volume of ejaculate, normal sperm and concentration of sperm by 0.14 ml, 2.2% and $45 \times 10^6/ml$, respectively. While the TC genotype was

higher than TT and CC genotypes for *pH* of semen, motility of sperms and live sperms with an increase by 0.2, 3.5% and 3.3%, respectively, expect the TT genotype was the lowest in dead sperms and abnormal sperms and with a decrease by 3.1% and 1.9%, respectively.

- In Moshtohor line rabbits, the differences among TT, TC and CC genotypes of *GH* gene in most semen traits were significant ($P < 0.05$). The TC genotype was the highest in *pH* of semen, motility of sperms, live sperms, normal sperms and concentration of sperms and with an increase by 2, 3.1%, 2.5%, 1.7% and $20.8 \times 10^6/ml$, respectively, while the CC genotype was the highest in volume of ejaculate and with an increase by 2 *ml*, expect the TT genotype was the lowest in dead sperms and abnormal sperms and with decrease by 1.8% and 2.5%, respectively.
- In APRI line rabbits, the differences among TT, TC and CC genotypes of *GH* gene were significant for all semen traits ($P < 0.05$). The CC genotype was the highest in volume of ejaculate, *pH* of semen, and concentration of sperms and with an increase by 1.3 *ml*, 2.0 and $232.2 \times 10^6/ml$, respectively and TC genotype was the highest in motility of sperms, live sperms and normal sperms, with an increase by 2.1%, 2.3% and 2.7%, respectively, expect the TT genotype was the lowest in dead sperms and abnormal sperms with a decrease by 5% and 5.2%, respectively.
- In $\frac{1}{2}A\frac{1}{2}M$ cross rabbits, the differences among TT, TC and CC genotypes of *GH* gene were significant in all semen traits ($P < 0.05$). The TT genotype was the highest in volume of ejaculate, *pH* of semen, motility of sperms, live sperms, normal sperms and

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concentration of sperms, with an increase by 0.1 *ml*, 0.2, 3.6%, 4.2%, 1.0% and $97.9 \times 10^6/ml$, respectively, while the CC genotype was the lowest in dead sperms and abnormal sperms and with a decrease by 4.3% and 1.0%, respectively.

- In Sinai Gabali breed rabbits, the differences among TT, TC and CC genotypes of *GH* gene were significant in all semen traits ($P < 0.05$). The TT genotype was the highest in *pH* of semen, motility of sperms, normal sperms and concentration of sperms, with an increase by 0.1, 8.7%, 0.7% and $95.1 \times 10^6/ml$, respectively, while the TC genotype was the highest in volume of ejaculate, live sperms and with an increase by 0.24 *ml* and 2.6%, respectively, expect the CC genotype was the lowest in dead sperms and abnormal sperms, with a decrease by 2.5% and 0.6%, respectively.

CONCLUSIONS

- ❖ Crossing APRI line with Moshtohor line was associated with heterotic effects and could be beneficial to produce rabbits characterized by heavy body weights and gains and bucks with high quality semen traits.
- ❖ Based on direct and maternal effects, APRI line could be used as a sire-group and Moshtohor line as a dam-group in synthesizing new line of rabbits in Egypt to improve growth traits and quality of semen traits.
- ❖ The PCR-RFLP technique is an appropriate tool for screening the genotypes of *GH* gene and for evaluating the genetic variability in rabbit's breeds.
- ❖ The significant associations between *GH* gene and growth traits confirmed that this gene could be was as a candidate gene and may be used as Marker-assisted selection in genetic improvement programs (MAS) to improve growth performance in rabbits and enhance the semen traits in the Egyptian rabbits.

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الملخص العربي

أجريت تجربة خلط بسيط بين ذكور الأرانب من خط الأبري (A) مع إناث من خط مشتهر (M)، وهما سلالتين مستنبطتين حديثاً في مصر لإنتاج اللحم. تم تأسيس خط الأبري في المحطات التجريبية التابعة لمعهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، وزارة الزراعة، مصر. بينما تم تأسيس خط مشتهر في كلية الزراعة، جامعة بنها، مصر. تم إجراء خطة الخلط خلال موسمين إنتاجيين متتاليين (2015-2016، 2016-2017) بمزرعة الأرانب، قسم الإنتاج الحيواني، كلية الزراعة، جامعة بنها. أجريت التحليلات الجزيئية بالمعمل المركزي، كلية الطب البيطري، جامعة بنها. وكانت الاهداف الرئيسية لهذه الدراسة هي: (1) تقدير مكونات التباين، المكافئ الوراثي، الأثر الوراثي التجمعي، الأثر التجمعي الأمي، قوة الخلط المباشرة لصفات وزن الجسم (عند عمر 4، 6، 8، 10، 12 أسبوع) ومعدل الزيادة اليومية بعد الفطام (خلال الفترات من 4-6، 6-8، 8-10، 10-12 أسبوع)، وصفات جودة السائل المنوي (حجم القذف المنوي – درجه تركيز أيون الهيدروجين – حركة الحيوانات المنوية – تركيز الحيوانات المنوية – نسبة الحيوانات المنوية الحية والميتة – نسبة الحيوانات المنوية الطبيعية والشاذة)، (2) استخدام تقنية PCR-

RFLP لمعرفة التنوع الجزيئي لجين هرمون النمو في خمسة

مجاميع وراثية مختلفة هي خط أبري (A)، خط مشتهر (M)، $1/2A1/2M$ ، V line، سلالة الجبلي السيناوي، (3) الكشف عن الارتباطات الجزيئية التنوعية للتراكيب الوراثية المختلفة لجين هرمون النمو مع صفات النمو وصفات جودة السائل المنوي. تم استخدام عدد 1201 أرنب مفطوم نتجت من 179 أب، 261 أم لإجراء التحليلات الكمية والجزيئية لدراسة صفات النمو، بينما تم استخدام عدد 1050 قذفة منوية والتي تم جمعها من 149 ذكر لدراسة صفات جودة السائل المنوي. استخدمت طريقة المربعات الصغرى المعممة (GLS) Generalized Least Squares لتحليل البيانات إحصائياً. يمكن تلخيص أهم نتائج هذه الدراسة على النحو التالي:

التحليلات الوراثية الكمية لصفات النمو وصفات جودة السائل المنوي:

- كانت قيم المكافي الواثي لصفات وزن الجسم ومعدل الزيادة اليومية منخفضة إلى متوسطة غالباً وتراوح من 0.06 إلى 0.18 للقيم المتحصل عليها بإستخدام برنامج VCE ومن 0.09 إلى 0.18 للقيم المتحصل عليها بإستخدام برنامج (TM) Threshold Model. كانت قيم المكافي الوراثي لصفات جودة السائل المنوي والمتحصل عليها بإستخدام برنامج TM منخفضة جداً وتراوح من 0.01 إلى 0.03، بينما كانت متوسطة نسبياً وتراوح من 0.12 إلى 0.20 للقيم المتحصل عليها بإستخدام برنامج VCE.
- كانت تقديرات الأثر الوراثي التجمعي معنوية لصالح أرناب خط مشتهر بمعدل 13.5 إلى 48.1 جرام لصفات وزن الجسم وبمعدل 0.5 إلى 3.7 جرام لصفات معدل الزيادة اليومية في الوزن، وتراوح النسب المئوية للأثر الوراثي التجمعي من 1.4 إلى 4.3% لصفات وزن الجسم، ومن 2.1 إلى 18.6% لصفات معدل الزيادة اليومية. وكانت تقديرات الأثر الوراثي التجمعي معنوية وفي صالح خط مشتهر لصفات جودة السائل المنوي بمعدل 0.04 ملي، 0.2، 0.4%، $20.5 \times 10^6/ml$ لصفات حجم القذف المنوية، تركيز أيون الهيدروجين، نسبة الحيوانات المنوية الشاذة وتركيز الحيوانات المنوية على الترتيب وبنسب مئوية تراوحت من 2.6 إلى 5.3%.
- كانت تقديرات الأثر التجمعي الأمي معنوية لصالح أرناب خط مشتهر وبمعدل 14.2 إلى 51.8 جرام لصفات وزن الجسم وبمعدل 0.48 إلى 3.1 جرام لمعدلات الزيادة اليومية في الوزن، وتراوح النسب المئوية للأثر الوراثي الأمي من 2.8 إلى 5.4% لصفات وزن الجسم، ومن 1.8

إلى 15% لصفات معدل الزيادة اليومية. وكانت تقديرات الأثر التجمعي الأمي معنوية لصفات جودة السائل المنوي لصالح خط مشتهر بمعدل 0.05 ملي، 0.4، 3.3%، 1.2%، 0.7%، $22.8 \times 10^6 / ml$ لصفات حجم القذفة المنوية، تركيز أيون الهيدروجين، حركة الحيوانات المنوية، نسبة الحيوانات المنوية الميتة، نسبة الحيوانات المنوية الشاذة وتركيز الحيوانات المنوية على الترتيب وبنسب مئوية تراوحت من 1.2 إلى 8.1%.

- كانت تقديرات قوة الخلط المباشرة موجبة وعالية المعنوية وتراوحت من 15.5 إلى 87.1 جرام لصفات وزن الجسم ومن 1.6 إلى 2.79 جرام لصفات معدل الزيادة اليومية في الوزن، وتراوحت النسب المئوية لقوة الخلط المباشرة من 3.1 إلى 8.2% لصفات وزن الجسم ومن 1.1 إلى 13% لصفات معدل الزيادة اليومية. وبالنسبة لصفات جودة السائل المنوي كانت تقديرات قوة الخلط المباشرة مصحوبة بتحسين في حجم القذفة المنوية، تركيز أيون الهيدروجين، حركة الحيوانات المنوية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية الميتة، نسبة الحيوانات المنوية الشاذة وتركيز الحيوانات المنوية بمعدل 0.09 ملي، 0.7، 5.8%، 3.1%، 3.3%، 1.1%، $30.7 \times 10^6 / ml$ وتراوحت النسب المئوية لقوة الخلط المباشرة من 3.8 إلى 16.9%.

التنوع الجزيئي لجين هرمون النمو في المجاميع الوراثية المختلفة:

- عبر كل المجاميع الوراثية المختلفة كان تكرار التركيب الوراثي TT لجين هرمون النمو عالي المعنوية وتراوح من 0.48 في V line تليها سلالة الجبلي (0.39)، M line (0.32)، A line (0.31) وخليط الجيل الأول $1/2A \times 1/2M$ (0.28). بالنسبة للتركيب الوراثي CC سجل M line أعلى التكرارات معنوية (0.21)، بينما سجل خليط الجيل الأول $1/2A \times 1/2M$ أقل

التكرارات (0.04)، وبالنسبة للتركيب الوراثي TC كان أعلى تكرار معنوي له في خليط الجيل الأول $1/2A1/2M$ (0.67) وأقل تكرار له في V line (0.45). سجل M line أعلى تكرار للأليل C (0.45) بينما كان أقل تكرار لهذا الأليل في سلالة الجبلي (0.32).

- سجل M line أعلى قيمة لعدد الأليلات الفعال للتركيب الوراثي TC (1.978) يليه خط أبري (1.899) ثم خليط الجيل الأول $1/2A1/2M$ (1.893)، بينما كانت أقل قيم لعدد الأليلات الفعال في V line (1.715) وسلالة الجبلي (1.800).

- كانت الإنحرافات عن إتزان هاردي-فاينبرج غير معنوية في جميع العشائر المدروسة.

- كانت قيم التراكيب الوراثية الخليطة المشاهدة (H_o) أعلى من قيم التراكيب الوراثية الخليطة المتوقعة (H_e) في كل المجاميع الوراثية، وتراوحت القيم المتوقعة من 0.444 في V line إلى 0.667 في خليط الجيل الأول $1/2A1/2M$. بينما تراوحت القيم المشاهدة من 0.425 في V line إلى 0.508 في M line.

- كانت كل قيم محتوى المعلومات للتنوع الجزيئي (PIC) متوسطة وسجلت 0.332، 0.375، 0.360، 0.360، 0.341 في V line، M line، A line، $1/2A1/2M$ وسلالة الجبلي على الترتيب.

- كان النقص في Heterozygosity (F_{IS}) نتيجة التربية الداخلية لكل موقع وراثي عبر الخمسة مجاميع وراثية محل الدراسة متوسط أو منخفض وسجل أعلى قيمة له في M line (0.042) وأقل قيمة له في خليط الجيل الأول $1/2A1/2M$ (-0.413).

- كانت مستويات التنوع الوراثي عبر الخمسة مجاميع وراثية المدروسة متوسطة ($F_{IS} = -0.198$ ، $PIC = 0.358$ ، $H_e = 0.471$ ، $H_o = 0.551$).

الارتباطات الجينية التنوعية بين التراكيب الوراثية لجين هرمون النمو

وصفات النمو:

- في أرناب V line، غاب التركيب الوراثي CC وكانت الارتباطات بين التراكيب الوراثية TT، TC معنوية مع صفات النمو، حيث سجل التركيب الوراثي TT أقل ارتباطات عن التركيب الوراثي TC في أوزان الجسم عند عمر 6، 8، 10، 12 أسبوع. إرتبط التركيب الوراثي TC بزيادة موجبة ومعنوية في وزن الجسم عند عمر 6، 8، 10، 12 أسبوع بمعدل زيادة مقداره 32، 9، 57، 108 جرام على الترتيب. بالنسبة لمعدل الزيادة اليومية، غاب التركيب الوراثي CC وكان التركيب الوراثي TT أقل زيادة يومية مقارنة بالتركيب الوراثي TC، كما كانت الاختلافات بين التراكيب الوراثية معنوية لمعظم صفات معدل الزيادة اليومية في الوزن.
- في أرناب M line، كانت الاختلافات بين التراكيب الوراثية المختلفة لجين هرمون النمو معنوية، حيث كان التركيب الوراثي TC أثقل وزناً لصفات وزن الجسم عند عمر 4، 6، 8، 10، 12 أسبوع مقارنة بالتراكيب الوراثية TT، CC. إرتبط التركيب الوراثي TC بزيادة موجبة ومعنوية في وزن الجسم عند عمر 4، 8، 10، 12 أسبوع بمعدل زيادة مقداره 134، 119، 636، 734 جرام على الترتيب. كانت الاختلافات بين التراكيب الوراثية المختلفة لجين هرمون النمو معنوية لكل صفات معدل الزيادة اليومية خلال الفترات 6-8، 8-10، 10-12 أسبوع وكان التركيب الوراثي TT أقل وزناً من التراكيب الوراثية TC، CC.
- كانت الاختلافات بين التراكيب الوراثية المختلفة لجين هرمون النمو في A line معنوية، حيث كان التركيب الوراثي CC أثقل وزناً عند عمر 4، 10 أسابيع عن التراكيب الوراثية TC، TT، بينما كان التركيب الوراثي TC مرتبطاً بزيادة موجبة في وزن الجسم عند عمر 6، 8، 12 أسبوع بمعدل

43، 61، 305 جرام على الترتيب. كانت الاختلافات بين التراكيب الوراثية المختلفة لجين هرمون النمو معنوية لصفات معدل الزيادة اليومية خلال الفترات 6-8 ومن 10-12 أسبوع من العمر.

- كانت الاختلافات بين التراكيب الوراثية المختلفة لجين هرمون النمو في خليط الجيل الأول $1/2A1/2M$ معنوية لكل صفات وزن الجسم عدا وزن الجسم عند عمر 4 أسابيع، كما غاب التركيب الوراثي CC. كان التركيب الوراثي TT أثقل وزنا من التركيب الوراثي TC في جميع صفات وزن الجسم، وإرتبط التركيب الوراثي TT بزيادة موجبة بمعدل زيادة مقداره 22، 6، 88، 29 جرام لصفات وزن الجسم عند عمر 6، 8، 10، 12 أسبوع على الترتيب. بالنسبة لصفات معدل الزيادة اليومية في الوزن، كانت الاختلافات بين التراكيب الوراثية المختلفة لجين هرمون النمو في خليط الجيل الأول $1/2A1/2M$ معنوية، وكان التركيب الوراثي TT أعلى في كل صفات معدل الزيادة اليومية مقارنة بالتركيب الوراثي TC، كما غاب التركيب الوراثي CC، وكان التركيب الوراثي TC أثقل وزنا من التركيب الوراثي TT في معدل الزيادة اليومية في الوزن خلال الفترة من 8-10 أسبوع.

الارتباطات الجزيئية التنوعية بين التراكيب الوراثية لجين هرمون النمو

وصفات جودة السائل المنوي:

- في أرناب V line، كانت الارتباطات بين التراكيب الوراثية TT، TC، CC لجين هرمون النمو معنوية مع بعض صفات جودة السائل المنوي مثل حجم القذفة المنوية، نسبة الحيوانات المنوية الطبيعية، تركيز الحيوانات المنوية. أظهر التركيب الوراثي TT قيم عالية مقارنة بالتراكيب الوراثية TC، CC مع زيادة في صفات حجم القذفة المنوية، نسبة الحيوانات المنوية الطبيعية، تركيز الحيوانات المنوية وذلك بمعدل زيادة مقداره 0.14 ملي،

2.2% ، 45×10^6 /ملي علي الترتيب. بينما كان التركيب الوراثي TC أعلى من التراكيب الوراثية TT، CC في صفات تركيز أيون الهيدروجين، حركة الحيوانات المنوية، نسبة الحيوانات المنوية الحية وكانت الزيادة بمعدل 0.2 ، 3.5% ، 3.3% علي الترتيب. وكان التركيب الوراثي TT أقل في نسبة الحيوانات المنوية الميتة وفي نسبة الحيوانات المنوية الشاذة مع الانخفاض في هذه الصفات بمعدل 3.1% ، 1.9% علي الترتيب .

- في أرناب M line، كانت الارتباطات بين التراكيب الوراثية TT، TC، CC لجين هرمون النمو ولمعظم صفات جودة السائل المنوي معنوية. وكان التركيب الوراثي TC غالب في صفات تركيز أيون الهيدروجين، حركة الحيوانات المنوية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية الطبيعية، تركيز الحيوانات المنوية مع الزيادة في هذه الصفات بمعدل 2.0 ، 3.1% ، 2.5% ، 1.7% ، 20.8×10^6 /ملي علي الترتيب، وكان التركيب الوراثي CC أعلى في صفات حجم القذفة المنوية مع الزيادة بمعدل 2.0 ملي. بينما كان التركيب الوراثي TT أقل في نسبة الحيوانات المنوية الميتة وفي نسبة الحيوانات المنوية الشاذة مع الانخفاض في هذه الصفات بمعدل 1.8% ، 2.5% علي الترتيب.

- في أرناب A line، كانت الارتباطات بين التراكيب الوراثية TT، TC، CC لجين هرمون النمو وكل صفات جودة السائل المنوي معنوية. وكان التركيب الوراثي CC أعلى في صفات حجم القذفة المنوية، تركيز أيون الهيدروجين، تركيز الحيوانات المنوية وكانت الزيادة بمعدل 1.3 ملي، 2.0% ، 232.2×10^6 /ملي علي الترتيب، وكان التركيب الوراثي TC أعلى في صفات حركة الحيوانات المنوية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية الطبيعية مع الزيادة بمعدل 2.1% ، 2.3% ، 2.7% علي الترتيب بينما كان التركيب الوراثي TT أقل في نسبة الحيوانات

المنوية الميتة وفي نسبة الحيوانات المنوية الشاذة مع الانخفاض في هذه الصفات بمعدل 5.0%، 5.2% علي الترتيب.

- في خليط الجيل الأول $1/2A1/2M$ ، كانت الارتباطات بين التراكيب الوراثية TT، TC، CC لجين هرمون النمو وكل صفات جودة السائل المنوي معنوية. وكان التركيب الوراثي TT أعلى في صفات حجم القذفة المنوية، تركيز أيون الهيدروجين، حركة الحيوانات المنوية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية الطبيعية، تركيز الحيوانات المنوية وبذلك كانت الزيادة بمعدل 0.1 ملي، 0.2، 3.6%، 4.2%، 1.0% 97.9×10^6 ملي علي الترتيب، بينما كان التركيب الوراثي CC أقل في صفات نسبة الحيوانات المنوية الميتة وفي نسبة الحيوانات المنوية الشاذة مع الانخفاض في هذه الصفات بمعدل 4.3%، 1.0% علي الترتيب.
- في أرناب الجبلي السيناوي، كانت الارتباطات بين التراكيب الوراثية TT، TC، CC لجين هرمون النمو وكل صفات جودة السائل المنوي معنوية. وكان التركيب الوراثي TT أعلى في صفات تركيز أيون الهيدروجين، حركة الحيوانات المنوية، ونسبة الحيوانات المنوية الطبيعية وتركيز الحيوانات المنوية مع الزيادة بمعدل 0.1، 8.7%، 0.7% 95.1×10^6 ملي علي الترتيب، بينما كان التركيب الوراثي TC أعلى في صفات حجم القذفة المنوية، نسبة الحيوانات المنوية الحية كانت الزيادة بمعدل 0.24 ملي، 2.6% علي الترتيب. بينما كان التركيب الوراثي CC أقل في صفات نسبة الحيوانات المنوية الميتة وفي نسبة الحيوانات المنوية الشاذة مع الانخفاض في هذه الصفات بمعدل 2.5%، 0.6% علي الترتيب.

تأثير الخلط علي بعض صفات السائل المنوي وخلفه البطن في الأرناب

رسالة مقدمة من

عبدالفتاح راشد عبدالفتاح زغلول
بكالوريوس في العلوم الزراعية (الإنتاج الحيواني) كلية الزراعة بمشتهر - جامعة
بنها

٢٠١٣

للحصول علي درجة
الماجستير في العلوم الزراعية
الإنتاج الحيواني (تربية الحيوان)

لجنة الإشراف:

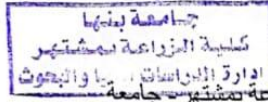
أ.د ماهر حسب النبي خليل.....
أستاذ تربية الحيوان المتفرغ - كلية الزراعة - جامعة بنها ومستشار رئيس
جامعة بنها للبحث العلمي.

أ.د محمود مغربي عراقي.....
أستاذ تربية الدواجن وعميد كلية الزراعة - جامعة بنها.

د شريف إبراهيم رمضان.....
أستاذ مساعد الإنتاج الحيواني والدواجن - كلية الطب البيطري - جامعة بنها.

٢٠١٩

تأثير الخلط علي بعض صفات السائل المنوي وخلفه البطن
في الأرناب



رسالة مقدمة من

عبدالفتاح راشد عبدالفتاح زغلول

بكالوريوس في العلوم الزراعية (الإنتاج الحيواني) كلية الزراعة جامعة أسيوط

٢٠١٣

أ. د. جاسم

للحصول علي درجة
الماجستير في العلوم الزراعية
الإنتاج الحيواني (تربية الحيوان)

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رسالة مقدمة من الطالب
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(تربية الحيوان)

من
قسم الإنتاج الحيواني
كلية الزراعة بمشتهر
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